The BIOCHEMISTRY of GASTRIC ACID SECRETION

EDWARD J. CONWAY, D.Sc., M.D.

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The BIOCHEMISTRY *of* GASTRIC ACID SECRETION

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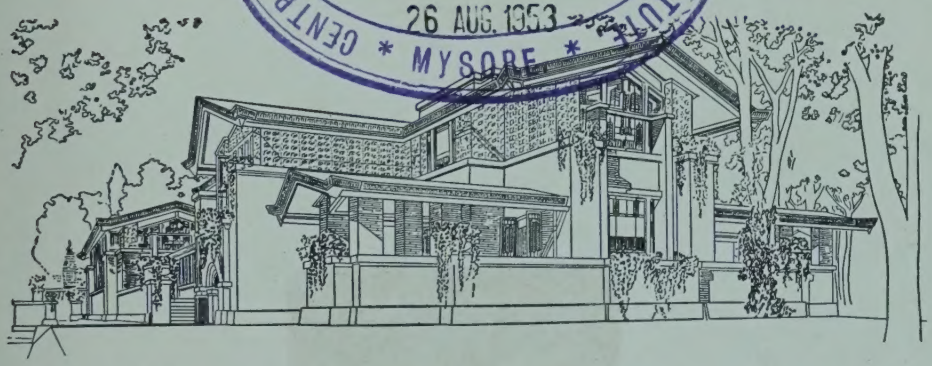

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To

F. G. DONNAN

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The BIOCHEMISTRY *of* GASTRIC ACID SECRETION

CHAPTER I

THE GASTRIC MUCOSA AND THE SITE OF THE ACID SECRETING CELLS

GENERAL DESCRIPTION

The stomach wall (Fig. 1) in man and higher animals is made up of a mucous layer or mucosa containing the secreting glands, a submucous and a muscular coat, outside which is a serous coat derived from the peritoneum.

The muscular coat, made of plain muscle fibre, consists typically of outer longitudinal, middle circular and internal oblique layers.

The submucosa consists of loose connective tissue containing blood vessels and lymphatics, and contains a ganglionated nerve plexus similar to the plexus of Meissner in the intestine.

The mucous or glandular coat is bounded externally by the muscularis mucosae which consists of an outer longitudinal and inner circular layer of plain muscular fibres, the inner layer sending strands of muscle towards the surface, between the glands. When the stomach is empty the muscular coats contract and the inextensible mucous membrane is thrown into numerous prominent folds. Its surface is mamillated or marked out into a number of small, slightly elevated polygonal areas. These little areas from 1 to 6 mm. in diameter have numerous small pits, *foveolae gastricae*, about 0.2 mm. wide, the surface separating them forming a series of elevated ridges, *plicae villosae*, resembling villi on section.

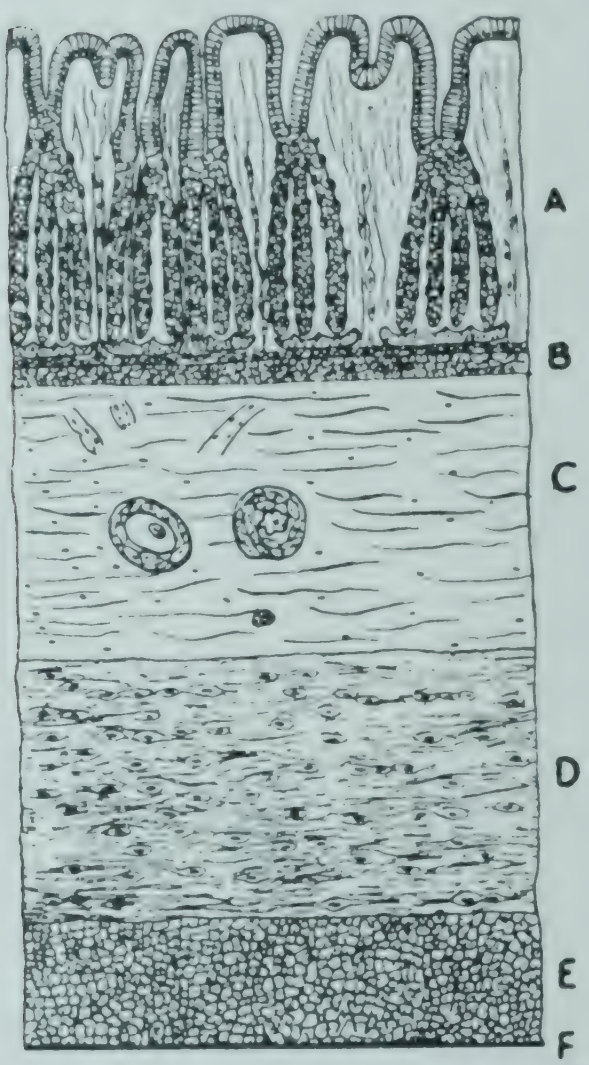

Fig. 1. Diagrammatic section through wall of stomach. (A) Mucous layer with glands opening into gland ducts. (B) Muscularis mucosae. (C) Submucosa. (D) Circular muscle layer. (E) Longitudinal muscle layer. (F) Serous coat.

The gastric glands. The gastric glands differ somewhat in different parts of the mucosa. Such parts have been distinguished as a small area near the oesophagus termed the cardiac region and near the duodenal entrance,

the pyloric region, the main part being termed the fundus or corpus. Often the stomach is considered simply as divided into two regions, a cardiac (including the fundus) and a pyloric region, and various other names have been used.

In general a gastric gland is composed of secreting tubules, one or more in number, which open at the surface into the foveola (crypt or pit). An average number of 4.3 tubules open into each crypt of the gastric mucosa of an adult man (Scott, 1925), and the average number of tubules in the human subject is nearly fourteen million (about half a million in the new born, Scott, 1925).

In man the thickness of the mucous layer varies between 0.5 to 1.5 mm. (Babkin, 1950) but in the pig it may reach to 3.5 mm. (Holter and Linderstrøm-Lang, 1934). The width of the body of the gland is generally less than 100 μ and the inner diameter of the collecting tubule less than 10 μ (Hollander, 1943).

The following types of cells may be distinguished.

(a) **Surface columnar epithelium**. This lines the crypts and forms the innermost layer of the gastric mucosa. The cells secrete mucus, from mucigen granules (Schaffer, 1927). The mucus forms a layer covering the surface of the stomach and mucus threads may be observed joining the mucous layer (Bucher, 1932) with the surface epithelium.

(b) **Central cells.** The epithelium of the secreting tubules is composed of two kinds of cells termed from their position, *central* and *parietal.* The central include the peptic or chief cells of the fundic glands, and also a type of cell which is found in the neck of the gland and stains with a Mallory stain like mucin-containing cells. These are often referred to as the mucoid cells of the neck.

(c) **Parietal cells.** These cells, which secrete the

Fig. 2. Part of tubule of fundus gland showing lumen and the secreting canaliculi of the parietal cells; also the chief or pepsin-secreting cells (after Zimmermann).

HCl of the gastric juice, are concentrated chiefly in the neck of the glandular tubule, the body of the tubule being composed mainly of peptic cells.

In the pyloric and cardiac region (as distinct from the fundus of the stomach) there are very few parietal cells.

(d) **Other cells, probably of mesodermal origin.** These are comparatively few in number and it is not known if they participate in any way in gastric secretion. Among these the chromaffin, argentaffin or Heidenhain cells (called also by many other names, Plenk, 1932, Masson, 1932) have attracted most attention. The granules in the cells have a strong affinity for chromium and silver salts. The argentiffin cells were first described by Heidenhain (1879) and are found in other parts of the gastro-intestinal tract.

THE PARIETAL CELL

In the body of the glandular tubule the parietal cells are separated from the lumen of the gland by the peptic cells, but are connected therewith by the cellular capillaries. In the neck of the gland they are most abundant and directly face the lumen (Bensley, 1932).

In stained sections across the glands, they appear at the periphery and hence the term parietal cells. These have also been known as *oxyntic cells* (after Langley 1881) from their generally believed association with the production of HCl.

The parietal cells are large, spheroidal cells with a round nucleus (Fig. 2, after Zimmermann, 1898). Their granules are smaller than those of the peptic cells, have a low refractive power, and do not decrease in number during secretion. These granules are now regarded as mitochondria (Plenk, 1932).

Lim and co-workers (Lim and Ma, 1926; Ma, Lim and Liu, 1927) state that in the active cells the granules accumulate at the periphery and leave the middle free.

The parietal cells contain intracellular capillaries or *canaliculi* (Fig. 2) firstly described by Müller (1892) and investigated by Golgi (1893), Zimmermann (1898), and others, and are observable in the resting as well as in the

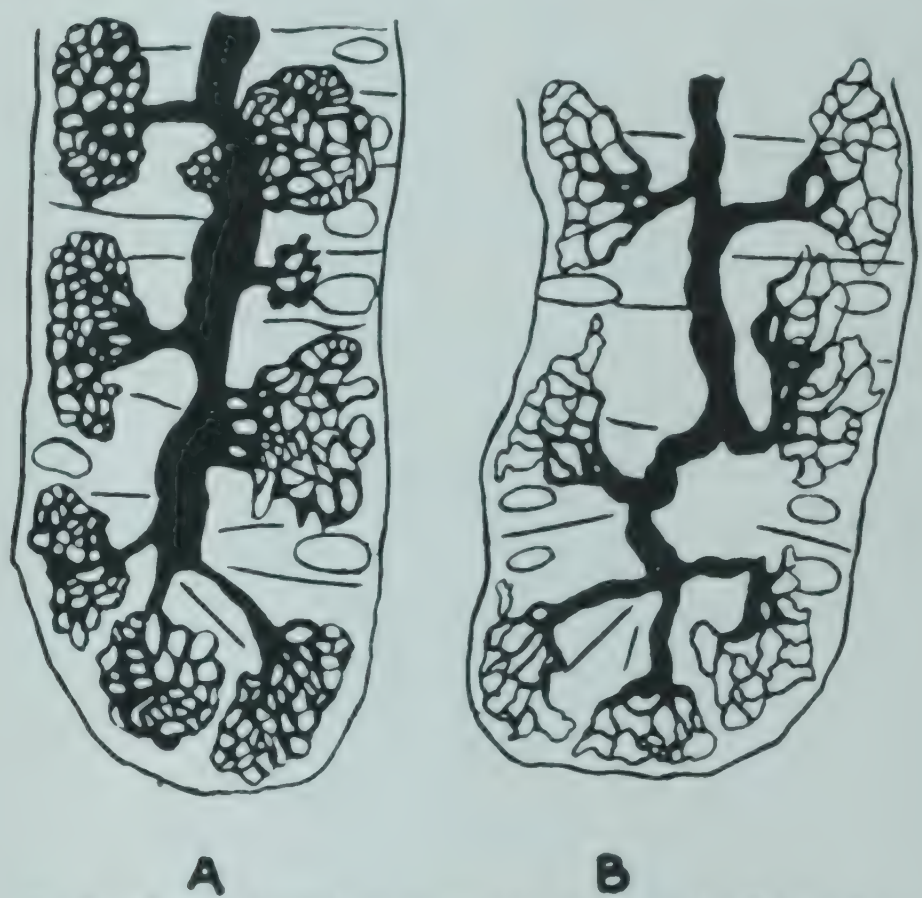

Fig. 2a. Fundus glands from rabbit stomach stained by Golgi's silver method showing the lumen and the canaliculi of the parietal cells. (A) Active cells; (B) Resting cells (after Golgi).

active cells. (Fig. 2a shows the canaliculi in resting and active cells, after Golgi.) Cowdry (1924) has observed that spirillae which normally inhabit the stomachs of the dog and cat may often be noticed within the gastric glands

and canaliculi of fasting subjects. Prussian blue can be deposited therein (FitzGerald, 1910) and various other dyes (Harvey and Bensley, 1912; Hoerr and Bensley, 1936). The intracellular capillary appears to have a distinct wall and there may be more than one outlet from the cell.

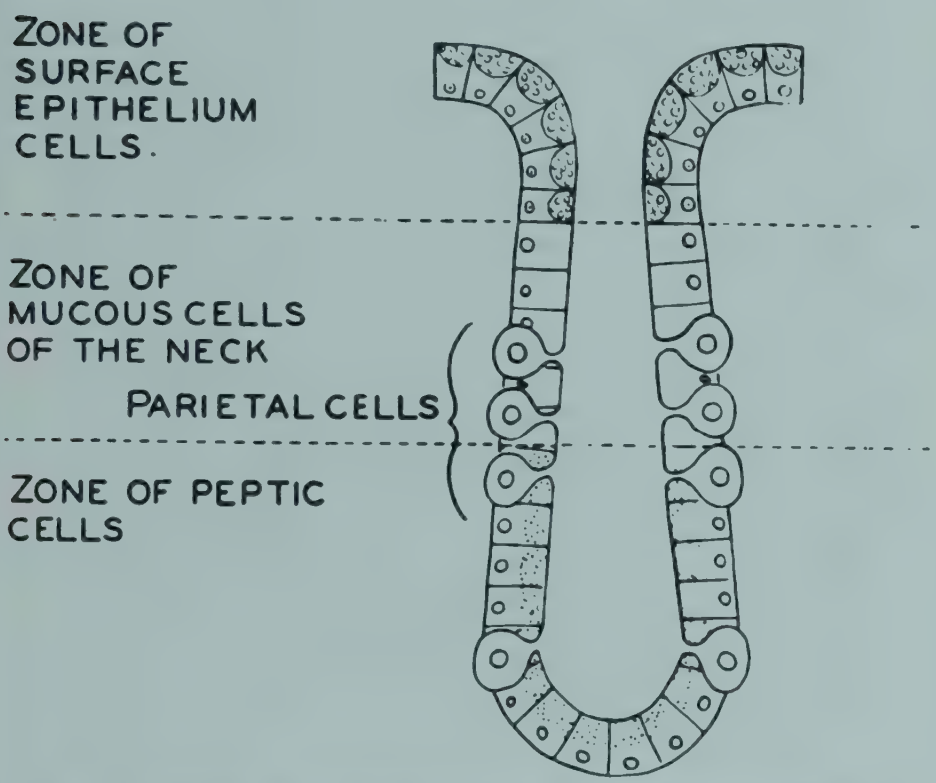

Fig. 3. Diagrammatic representation of cell distribution in a gastric gland (after Babkin, 1950). (Courtesy of *Canad. M. A. J.*)

Distribution of the parietal cells. In the glandular tubules they are mainly concentrated in the neck and to a much lesser extent in the body of the gland (Fig. 3).

Concerning the regional distribution in the mucosa, Figure 4 (after Berger, 1934) shows the relative amounts. In the pyloric region (white area) there is practically none.

In the hatched area of the cardiac region there is about 50 per cent and in the small cross hatched region at the

Fig. 4. Distribution of parietal cells (after Berger). Distribution in dark area taken as 100; in cross hatched it is then 75; in hatched 50 and 0 to 1 in white area (Berger, 1934; Courtesy of *Am. J. Anat.*)

incisura about 75 per cent of the number appearing in the fundus (dark region).

As the frog stomach has been much used in studying HCl formation it may be noted that the secretion of pepsin in the frog and related species occurs very largely

in glands in the oesophagus. The glands of the gastric mucosa secrete HCl and perhaps a little pepsin.

This was shown first by Swiecicki (1876) and later by Langley (1881). The acid-forming cells in the frog's stomach appear to be histologically similar to the parietal cells of the mammalian stomach and Langendorff and Laserstein (1894) have shown by the Golgi method, side branching from the lumen into these frog oxyntic cells, which seem to simulate in simpler form the canaliculi of the mammalian parietal cell. A drawing of a gland from the fundus region (after Langley) is shown in Figure 5.

Fig. 5. Drawing of gastric gland of frog (after Langley). E = surface epithelium. N = cells of neck. O = gland cells. (Courtesy of *Philos. Trans. Royal Soc.*)

THE PARIETAL CELL AS THE SITE OF HCl FORMATION

The parietal cells are regarded as the site of HCl formation.

The following evidence may be given for this conclusion:

1. The most important is provided by the work of Linderstrøm-Lang and co-workers (Holter and Linderstrøm-Lang, 1934, 1935; Glick, 1934, 1935; Linderstrøm-Lang, Holter and Ohlsen, 1934, 1935).

These investigated the micro-chemical distribution of HCl and various enzymes in the mucous membrane of the pig's stomach, proceeding from the mucosal surface towards the serosal. Gastric mucosa was removed, frozen at -10 to -11° C., achieving a suitable consistency thereby for the stamping out of small cylinders, 2.5 mm. in diameter. These were then sectioned in a freezing microtome, sections from 10 to 25 μ being cut parallel to the surface. Such sections were investigated for acid content by titrating with 0.01 M borate. They were also examined for their content in pepsin and other enzymes.

The mucosa adjacent to the sites from which the cylinders were taken was suitably fixed and stained and the different kinds of cells at various depths proceeding from the mucosal surface were enumerated.

The curves of acid content and parietal cell count against distance correspond. There is also a correspondence between pepsin content and number of chief cells. Figure 6 shows curves of HCl and pepsin distribution. Acid was found only in the mucous membrane of actively secreting stomachs.

When the diffusion conditions in such experiments are considered, it becomes a problem how any localisation of HCl is demonstrable. Thus the following equation may be applied (Conway, 1947)

$$\frac{100 - y}{100} = \frac{8}{\pi^2} e^{-(k\pi^2 t/4a^2)}$$

where a is the stratum depth at one end of which there is zero concentration, y is the percentage of the original concentration remaining as a whole in the stratum, t the time and k the diffusion coefficient; with a = 0.5 mm., and $k = 2.5 \times 10^{-3}$ (cm.^{2} min.) at room temperature (18° C) then in one minute approximately 90 per cent will have diffused and in two minutes about 99 per cent.

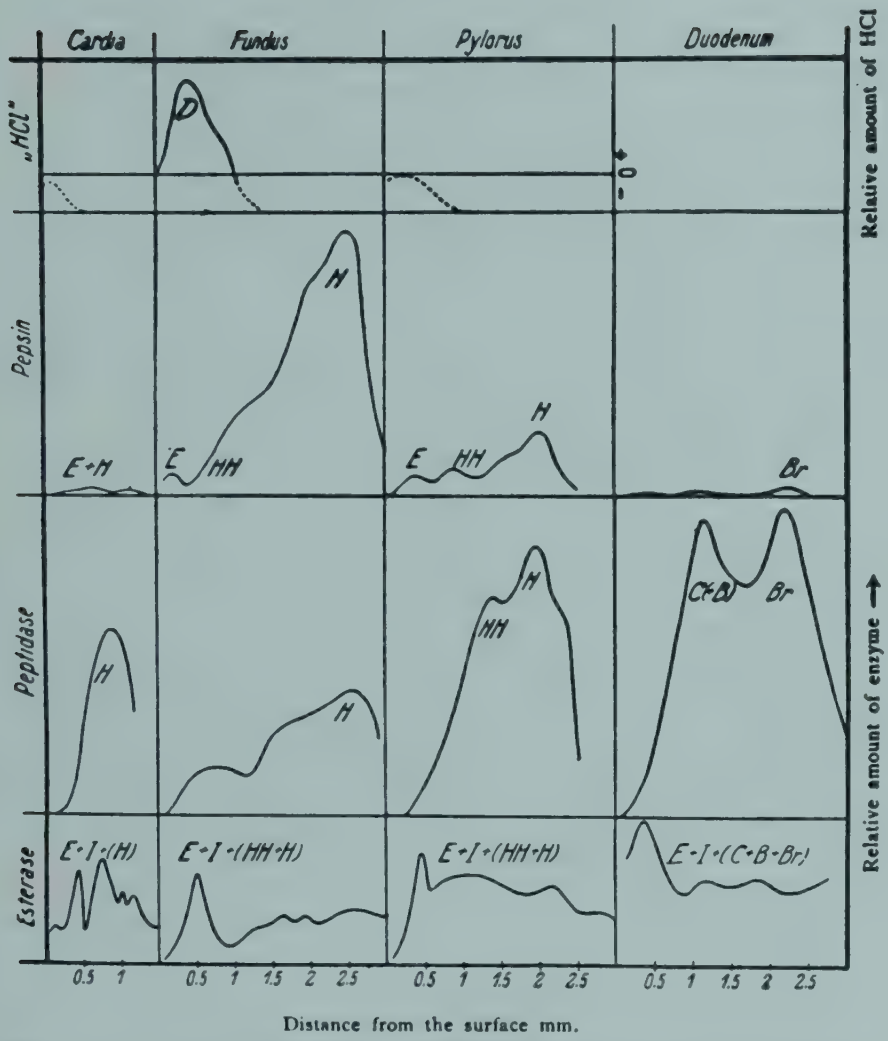

Fig. 6. Graphical summary of "HCl" and enzyme distribution in gastric and duodenal mucosa (after Glick, Holter, Linderstrøm-Lang and Ohlsen, 1935).

At zero centrigrade about double these times will give the order of figure. It will be seen that under the conditions the acid could be expected to have equalized in concentration throughout the tubule. Why then is it found localised in the parietal cell region?

The answer to this has been indicated by Davenport (1943). When the tissue is excised secretion stops at once (Dawson and Ivy, 1926), and it may also be assumed that the permeability of the canaliculi walls rapidly change. HCl diffuses then quickly into the parietal cells and is there retained owing to the low H ion concentration, but it can be titrated using a suitable pH value for the end point.

From such experiments the conclusion may be drawn that parietal cells are in all probability the cells which produce hydrochloric acid.

2. The evidence from pigments and histological examination appeared at first from the use of the Prussian blue reaction (Claude Bernard, 1859; Lepine, 1872; Sehrwald, 1889; Macallum, 1908; FitzGerald, 1910; Hammet, 1915; Harvey and Bensley, 1912; and Collip, 1920, who as well as Dawson and Ivy, 1926, strongly criticised the method); then from the use of a dye stuff whose colour varies with pH (Edinger, 1880, 1882; Trinkler, 1885; Stintzing, 1889; Frankel, 1891; Harvey and Bensley, 1912; Collip, 1920; Dawson and Ivy, 1926; Hoerr and Bensley, 1936); lastly from the investigation of the localisation of chloride by silver deposition and the effect of light (Greenwood, 1884; FitzGerald, 1910; Monti, 1913; Leschke, 1915; and Collip, 1920).

FitzGerald (1910) has reviewed the early literature of the Prussian blue reaction. It was first introduced by Claude Bernard (1859) for such investigations. He injected a solution of lactate of iron and one of potassium ferrocyanide into a jugular vein of a semi-fasting rabbit. Three-quarters of an hour later the animal was killed. A deposit of blue pigment was found on the surface of the mucosa, more particularly in the region of the lesser curvature, but no evidence of a similar deposit was found in the gland tubules.

Sehrwald (1889) criticised Claude Bernard's technique, pointing out that Prussian blue could not result directly from iron lactate which contained divalent iron, but because of oxidation. He used iron lactate with potassium ferricyanide which on acidification forms Turnbull's blue. However, his procedure of placing small sections of a fresh stomach for one day in lactate of iron would now be considered to give results of no value.

FitzGerald used the Prussian blue reaction avoiding some objections by the use of ammonium-ferric citrate and potassium ferrocyanide, injected in a balanced solution subcutaneously, or intravenously.

In the rabbit showing the most uniform results in all eight injections were given over about 30 hours. On killing the animal there appeared a curious localised formation of Prussian blue near the oesophagus and lesser curvature. In vertical sections of the mucosa of the stomach fixed in absolute alcohol the deposit appeared in the lumen of the upper third of the tubule but never in the lower third. It was often observed in the canaliculi of the parietal cells.

It is surprising, after all, from the diffusion conditions dealt with above, how well this accords with the view that acid is secreted by the parietal cells and into the canaliculi when we consider the small distances involved, and therefore the comparative rapidity of diffusion as discussed above. The diffusion effect is probably much less when deposition has occurred.

Hollander (1943) points out that owing to the opportunity for diffusion and neutralisation, "the presence of an unquestionably acid reaction of suitable low pH may constitute good evidence for the local existence of free acid, but the absence of such a reaction is utterly without significance." Davenport (1943) briefly reviewing this

literature considers that the best work (Dawson and Ivy, 1926) has shown only that a minute after cessation of secretion the parietal cells are more acid than pH = 6.8.

Histological observations of silver chloride deposition (FitzGerald, 1910; Collip, 1920; Monti, 1913; and Leschke, 1915), have shown that the parietal cells and intercellular canaliculi during activity have much more chloride than the body chief cells. On the other hand this regional difference is not supported by some observers, notably Gersh (1938).

3. Evidence of the following kind may also be cited. Two types of gastric secretion may be observed, one after histamine stimulation, and the other after vagal stimulation. In the first type pepsin is practically absent, and the secretion contains a high degree of acidity and very little organic matter. The other is rich in pepsin and contains much organic matter. With the latter secretion there is a marked diminution of the granules in the body chief cells (Bowie and Vineberg, 1935; Bowie, 1936), but this does not occur with the purely acidic secretion. The secretion of acid is therefore either from the parietal cells, the surface epithelium, or the mucoid cells of the neck of the gland (the argentaffin cells may be outruled, being few in number and distributed throughout the intestine).

Since the surface epithelial cells are certainly associated with mucus secretion and the same may be assumed for the mucoid cells, this leaves the parietal cells as the probable site of the acid secretion.

THE WALL OF THE PARIETAL CANALICULI AS THE SITE OF THE ACID FORMATION

The foregoing evidence as a whole leads to the conclusion that the parietal cells are responsible for the secretion

of HCl and this is now the accepted view. The evidence seems to point further to the view that the wall of the parietal canaliculi is probably the site of the acid formation. This was suggested as a possibility by Collip (1920) and by Dawson and Ivy (1926) and supported by Hollander (1943) in a review of the evidence.

The following considerations may be advanced in support:

1. The canaliculi of the parietal cells draining into the intercellular canaliculus form a remarkable drainage system for the cell. As noted by Hollander (1943), Golgi (1893), Langendorff and Laserstein (1894), as well as Hoerr and Bensley (1936), have reported that the canaliculi are swollen during active secretion, and practically collapsed in the fasting animal.

2. Following the work of Bulger, Allen and Harrison (1928) it was shown by Hanke (1937) and by Hanke, Johannsen and Hanke (1931) that for each equivalent of HCl secreted into the stomach, an equivalent of alkali appeared to have been liberated into the blood. For the isolated frog stomach, following Delrue (1935), this equivalence was made very probable by Davies (1948), but the complete proof appears to have been furnished by Teorell (1950). Also for the yeast cell, it was shown (Conway, Brady and Carton, 1949, 1950) that, for the conditions investigated when this secretes acid into the suspending fluid, a corresponding equivalent alkalinisation occurs within the cell.

This acid secretion by yeast can, under certain conditions, rather closely simulate the parietal secretion (as will be shown in Chapter III), and the H^+ ion formation in yeast occurs in the cell wall (Conway and Downey, 1948, 1950).

3. Histological evidence (see Fig. 16) points to a definite wall to the canaliculi.

SUMMARY

A short account of the histology of the gastric mucosa with reference to the secretion of acid has been given, along with the main evidence for concluding that the parietal cells are responsible for the HCl secretion. It is also considered that the wall of the parietal canaliculi is probably the site of the H^+ ion secretion.

CHAPTER II

THE PARIETAL SECRETION

From the evidence reviewed in the previous chapter it will appear that the HCl of the gastric juice is secreted by the parietal cells.

Pure parietal secretion free from *any* admixture with other secretion from the gastric glands has not been obtained, but inferences can be drawn as to its composition from the changes of composition of the gastric juice on varying the rate of secretion.

It was observed by Pavlov (1910) that the acidity of the gastric juice increased as the rate of secretion increased. This in itself is an unexpected result for a substance secreted at a higher chemical potential than that in blood plasma. Thus it is the opposite of what happens in the kidney secreting urea and other metabolites, or sodium chloride when this appears in the urine in higher concentration than in blood plasma.

Pavlov interpreted such and other facts by the theory that the gastric glands always secreted HCl at a high and constant concentration (a view previously suggested by Heidenhain, 1879). This secretion was then neutralised or diluted in varying degrees by the secretion of mucus, the effect being most pronounced at the beginning and end of a secretory phase.

Rosemann (1907, 1920) considered also this relation of rate to acidity but interprets it differently, namely as a constant chloride secretion by gastric glands isosmotic

with the blood, but with the cation components, Na^+ and H^+ ions in *variable proportion*, the relative amount of H^+ ions depending on the intensity of the secretory stimulation. In this Rosemann has been supported by MacLean and co-workers (1928, 1929) and in modified form by Katsch (1926).

FURTHER DEVELOPMENT OF THE PAVLOV THEORY

Instead of regarding the whole of the gland tubules as a single functional unit, as in the theories of Pavlov or of Rosemann, to-day the acidic secretion is considered to come from the parietal cells, with a non-parietal secretion in small amounts from the chief cells, mucoid cells of the neck and mucus secretion from the surface (Hollander, 1931, 1932, and 1934, and Hollander and Cowgill, 1931).

Hollander systematically examined the relationships of rate, acidity, and chloride content of the gastric juice obtained from Pavlov pouch dogs, but with a modification of the Pavlov technique designed to diminish irritation to the mucosa and secure a greater supply of vagal fibres to the pouch. Collection of the juice was carried out through a catheter introduced only for the fraction of a minute, instead of using a catheter retained throughout the experiment.

In these experiments the findings of Pavlov were confirmed, the acidity rising sharply with the onset of secretion and when this rate fell off the concentration of acid also began to fall. If, however, maximal doses of histamine were administered (0.4 mg./kg.) the acidity rose promptly to plateau values, the concentration not falling as the secretion rate declined towards zero at the end.

In this latter type of experiment the mucus could be considered as effectively washed out on the one hand and,

due to the improvement in the experimental technique, lessened in amount by the avoidance of undue stimulation of the mucosa. In experiment B27 (Fig. 7) the mean pH was 0.89 ± 0.01 and the acid concentration 157 ± 7 mM.

Definite evidence against the validity of the Rosemann view was obtained by plotting the mean value of total acid against the total chloride. A linear relation resulted. There was no constancy in the chloride concentration, and

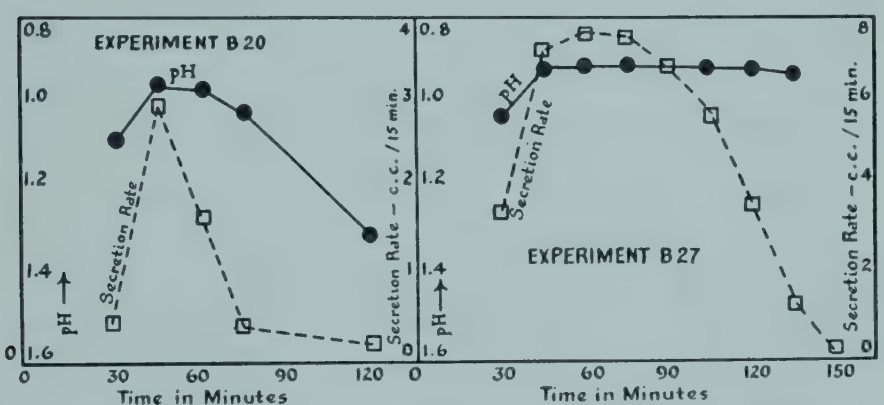

Fig. 7. Continuous collection experiments with histamine as stimulus. Experiment B20 was performed on a dog with a low secretory rate stimulated by 0.05 mg. of histamine per kg.; experiment B27 on a dog with a high secretory rate stimulated by 0.4 mg. of histamine per kg. (after Hollander, 1949; Courtesy of *Science*).

the lower chloride and acid values could be interpreted as arising from an alkaline non-parietal secretion low in chloride. Thus the mucous secretion would appear to contain bicarbonate as well as chloride. Such findings may be explained also in harmony with Teorell's "diffusion theory" (as considered in Chapter X).

Hollander considered the *parietal* secretion to be HCl free from neutral chloride and about 170 mM. The *non-parietal* secretion was made up of mucus and mucoid

fluid, and what is equivalent to a lymph transudate. It was also isotonic with the blood. Its chloride content was about 100 mM. with the remaining anions made up of bicarbonate, proteins and phosphate. He gives reviews in *Gastroenterology* (1943) and *Science* (1949). Broadly similar views were advanced by Liu, Yuan and Lim (1934).

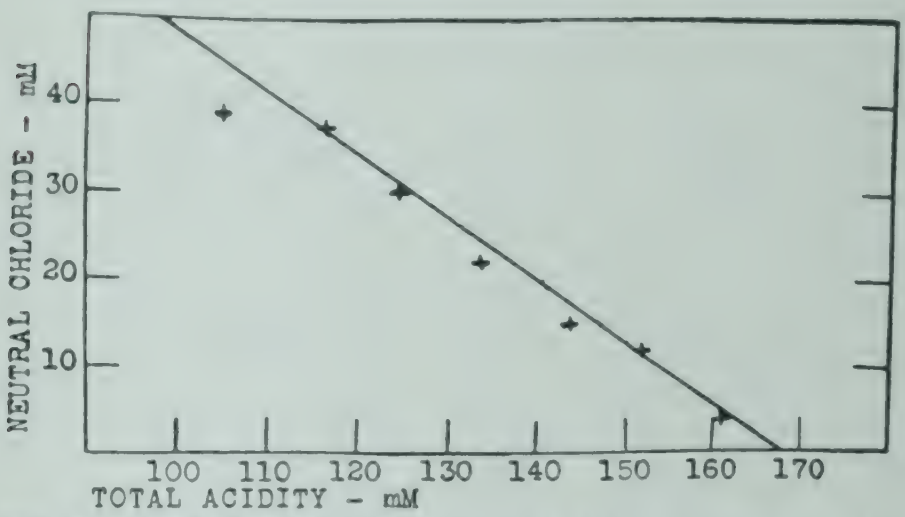

Fig. 8. Neutral chloride as a function of acidity; mean curve for 12 specimens obtained in 19 continuous collection experiments (after Hollander, 1949; Courtesy of *Science*).

Of interest for our present purpose is Hollander's graph of the neutral chloride against the total acid, as in Figure 8. When the line relating the points is extrapolated over the small distance in the figure, the value of the acid with no neutral chloride is reached, and the weighted mean is a 165 mM., pure hydrochloric acid solution.

This extrapolation, though over a small region, is liable to error as it assumes what should otherwise be proved, namely, the absence of neutral chloride in the purest secretion. For example, a small amount of inorganic cations

such as the K^+ ions may enter intrinsically into the secretory process (and from the yeast analogy, developed later, the significance of this will be more evident).

Gray (1943) examined the chemical analyses of 383 samples of gastric juice carefully collected from dogs with vagotomized pouches of the entire stomach (Gray and Bucher, 1941; Gray, Bucher and Harman, 1941). He found the maximum total chloride to be 166 m.eqs./l., which supported Hollander as against Liu, Yuan and Lim (1934) whose figure of 176 m.eqs./l. for the extrapolated value of the total acid was found too high.

When the mean value for the neutral chloride was plotted against the rate of secretion, it decreased markedly with the rate but the limiting value was not zero as Hollander supposed but a value as high as 12 m.eqs./l.

Analysis of other ions in the gastric juice showed that the K^+ ion concentration is constant at approximately 7 m.eqs./l. in spite of wide variations in acidity and rates of secretion (Fig. 9). In support Gray cites Gamble and McIver, 1928; Schairer, 1929; Bliss, 1930; Katsch, Baltzer and Brinck, 1934 (Linde and Öbrink 1950, report similar findings with Heidenhain pouch dogs).

From such studies Gray finally concludes that the parietal cell secretes an isotonic solution containing 166 m.eqs./l. of Cl^- ions, 159 m.eqs./l. of H^+ ion and 7 m.eqs./l. of K^+ ion.

Hollander (1949) mentions in answer to Gray's conclusions that they found neutral chloride concentrations as low as 3 to 5 mM. in several specimens which they analysed. This would not avoid the objection to extrapolation; further, the manner of determining this neutral chloride and its statistical range of error would need to be examined. Also for the data in Figure 9 Gray was dealing with the averages of large numbers of data, and

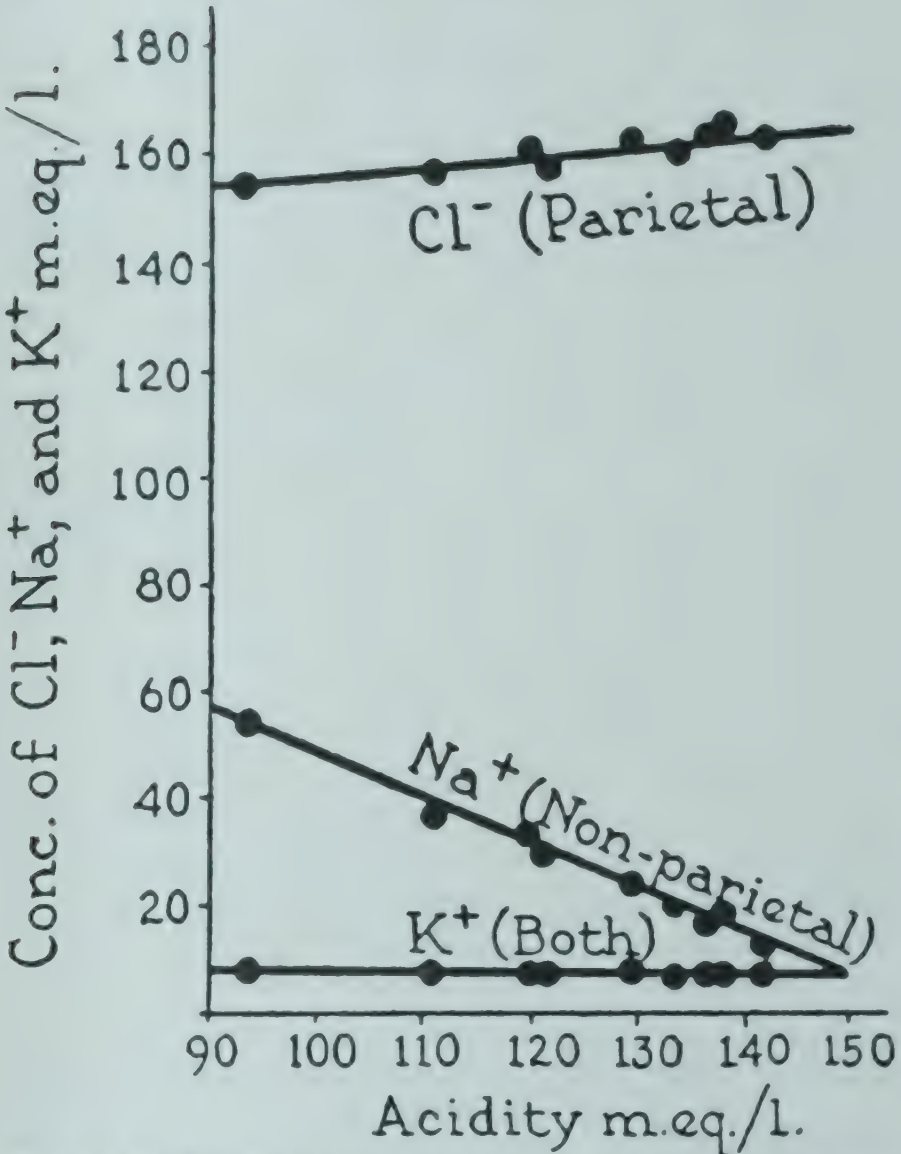

Fig. 9. Relationships between the concentrations of Cl^-, Na^+ and K^+ ions (after Gray, 1943; Courtesy of *Gastroenterology*).

though individual K data may be found to vary from the 7 mM. level, the evidence for the general validity of his conclusions appears strong.

Very recently Fisher and Hunt (1950) examined the

extensive data of Ihre (1939) on gastric secretion in selected young men in response to histamine and insulin administration, with a view to possible relations existing between acid, neutral chloride and volume of juice secreted.

They point out that Ihre's data are based on samples continuously withdrawn from the stomach; further that Ihre took precautions against contamination by saliva (using a saliva aspirator) and duodenal contents (continuous duodenal aspiration).

The initial assumptions made were that the gastric juice was a mixture of parietal and non-parietal secretions both of constant composition, the parietal containing HCl and neutral chloride, and the non-parietal bicarbonate and neutral chloride.

It was shown that the observations fitted the simple equations developed. By making the additional assumptions that the component secretions have the same osmotic pressure and that the common cation concentration is 170 m.eqs./l. the following estimates were made:

Parietal H^+ ion concentration.....160 m.eqs./l.
Parietal neutral Cl^- ion concentration....10 m.eqs./l.
Non-parietal Cl^- ion concentration.....125 m.eqs./l.
Non-parietal H CO_3^- ion concentration...45 m.eqs./l.

They consider it probable that the non-parietal secretion after histamine differs in composition from that after insulin. (Again it may be noted that such data can be interpreted apart from the assumption of a non-parietal bicarbonate secretion, as discussed in Chapter X.)

Concerning the potassium figure they consider that the lowest concentration recorded might be expected to give some indication of parietal component and cite that of 10 m.eqs./l. given by Katsch, Baltzer and Brinck (1934).

TEORELL'S GLYCINE EXPERIMENTS AND THE PRIMARY ACIDITY

Teorell showed (1939, 1940) that glycine is poorly absorbed in the stomach and, using 0.2 to 0.3 M. solution in the resting organ over relatively short periods, there was no appreciable volume change.

If a measured volume of such glycine solution be introduced into the stomach, the H ion concentration is maintained relatively low, and the possible diffusion exchange of Na^+ ions for H^+ ions (as in Teorell's "diffusion theory"; review by Öbrink, 1948) is thereby much reduced. According to Teorell this exchange accounts for the reduction in acidity of the pure gastric juice in the stomach and the increase of neutral chloride above that of the primary acidity.

By measuring the increase in acidity (by titration) and the corresponding volume increase, a figure for the primary acidity could be obtained. In this way Teorell (1940) showed that the HCl in narcotized cats appeared to be formed in higher osmotic concentration (208 ± 6 mN. HCl) than the blood. Later experiments were carried out on Heidenhain pouch dogs (Linde, Teorell and Öbrink 1947), receiving, in an unnarcotized condition, a continuous intravenous injection of histamine by which the secretion rate of HCl could be controlled to any desired level. The results obtained pointed towards the possibility that the primary acidity may have varying values between 170 mN. at the higher rates of secretion and approaching about 340–350 at the lower rates, these being the highest values actually observed, but an extrapolated value of 464 mN. was suggested.

Interpretation of Teorell's high values for the primary acidity. Linde, Teorell and Öbrink (1947) have themselves suggested that the buffer may possibly diffuse

back into the HCl-producing region, reduce the H^+ ion concentration there and result in new HCl production as a "compensating" mechanism. This would become prominent at the low rates of secretion and fade out as the secretion rate increased to high values.

Back-diffusion of the buffer must in fact occur, depending on the rate of secretion. With zero secretion rate and a diffusion distance of 0.5 mm., about 90 per cent of the total diffusion would occur in one to two minutes. With secretion rate V and a steady state arising, if a constant or near constant concentration at the mucosal end be assumed, the solution would have the form

$$C_A = C_0 e^{-\left(\frac{Vs}{k}\right)} \qquad (1)$$

where C_0 and C_A are the concentrations at each end, k is a constant which includes the diffusion coefficient, and s the distance. While histological data are inadequate for the precise application of such equations, the diffusion relations would appear in general sufficient for the following explanation.

EXPLANATION IN ACCORDANCE WITH A "REDOX" THEORY OF HCl PRODUCTION DISCUSSED LATER

Firstly, it is supposed that HCl is formed in the wall of the parietal canaliculi in a similar way to the formation of H^+ ions or of acid in the wall of the yeast cell. Its concentration during secretion increases to slightly hypertonic levels and water then diffuses outwards carrying the HCl in virtually isotonic solution into the lumen. If water is freely exchangeable across the wall of the canaliculi, as we may suppose, then the H^+ ion concentration will never be found significantly higher than about 170 mN.

in the gastric juice of the dog. Such would appear to be the underlying significance of the findings of Hollander, Gray and others, pointing to a parietal secretion, constant in concentration and isosmotic with the blood. If a buffer such as glycine is now introduced into the stomach, and diffuses back, then the H^+ ions secreted will be affected in the following way:

$$H^+ + Cl^- + \underset{\underset{NH_3^+}{|}}{CH_2\cdot COO^-} \rightarrow Cl^- + \underset{\underset{NH_3^+}{|}}{CH_2\cdot COOH}$$

where $\underset{\underset{NH_3^+}{|}}{CH_2\cdot COO^-}$ is the dipolar ion of glycine.

This reaction could go practically to completion. The glycine would exert the same osmotic pressure as before and the increase in the osmotically active ions would arise from chloride only and not from H^+ and Cl^- ions as in the absence of buffer. But if the volume increase is due to the rate of increase of osmotically active ions or molecules, then with a slow secretion rate in the presence of glycine:

$$\Delta(\text{HCl secreted})/\Delta V = \text{Total molar concentration of blood} = 340 \text{ mM}. \quad (2)$$

As the secretion rate increases less and less concentration of buffer will be present at the HCl production side so that the HCl will act osmotically more and more both as H^+ and Cl^- ions. In the limit:

$$2\Delta(\text{HCl secreted})/\Delta V = 340 \text{ mM}.$$

or

$$\Delta(\text{HCl})/\Delta V = 170 \text{ mM}. \quad (3)$$

Now the two limits at low concentration and at rapid secretion found by Linde, Teorell and Öbrink (1947) are

reported as 350 and 170, the difference from the above figures being scarcely significant. At the same time it may be added that they extrapolated the primary acidity values back to zero secretion rate, and reported a primary acidity then of about 464 mN., though Teorell has always advised caution in the interpretation of such values.

The extrapolation suffers from the double objection that the mathematical conditions chosen (rapid upward ascent of curve of primary acidity over very small range of secretion rate) can lead to a relatively large extrapolation error. Also, if the above explanation of the acidities be valid, no fall should occur until a certain critical flow is exceeded.

The empirical equation (which has the form of the diffusion equation 1) chosen to express their results is:

$$PA = PA_{\infty} + (PA_0 - PA_{\infty})e^{-cq} \qquad (4)$$

the PA symbols being the primary acidities at an observed rate q and at zero and infinite rates, c being a constant. It may be rearranged (including q_0 as the lower critical rate) as:

$$\log (PA - PA_{\infty}) = \log (PA_0 - PA_{\infty}) - c(q - q_0) \qquad (5)$$

expressing the logarithms, for convenience, to the base 10. As the value of PA_{∞} is not in doubt and may be written as 170 mN. then $\log (PA_0 - PA_{\infty})$ may also be regarded as a constant and is obtained (and hence PA_0) when the line of $\log (PA - PA_{\infty})$ against $(q - q_0)$ is extrapolated to zero value of $(q - q_0)$. This line is best determined as a statistical regression equation. Inserting the values of PA from Table II of Linde, Teorell and Öbrink's paper (1947) with $PA_{\infty} = 170$ mN., and using the figures above a q value of 0.5 ml./10 min. to avoid excessive variability, the following regression equation was determined:

$$\log (PA - PA_\infty) = -0.359q + 2.35$$
$$= -0.359(q - q_0) + (2.35 - 0.359q_0)$$

The regression line is shown in Figure 10 (correlation coefficient = −0.94). Neglecting q_0, the extrapolation to

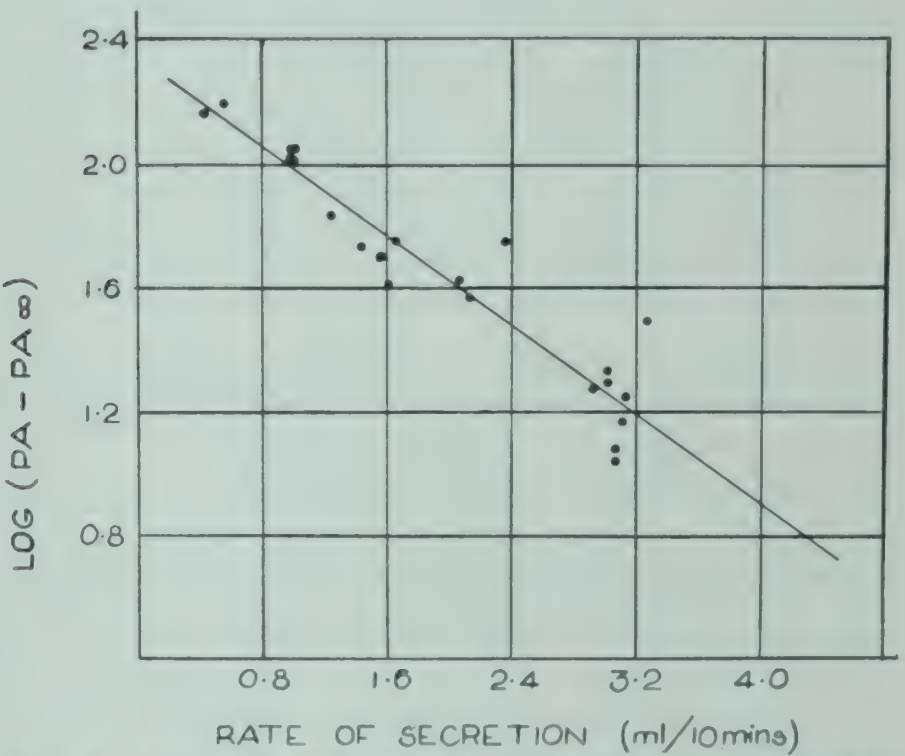

Fig. 10. From data of Linde, Teorell and Öbrink, obtained after introducing glycine solution into gastric pouch of dog and controlling secretion rate by intravenous histamine. PA = primary acidity at given rate of secretion; PA_∞ = primary acidity at very fast rate. Each point is the mean of the "direct" and "indirect" determinations. The regression equation is: $\log (PA - PA_\infty) = -0.359q + 2.35$.

zero rate would give a total PA_0 value of 393, but including allowance for q_0 it may be expected to be no more than 340 to 350 or near thereto, and so equal to the highest *observed* values of PA_0.

In the above interpretation of the primary acidity the

H^+ ion concentration at the point of secretion is never higher than about 170 mM., but the *sum* of the H^+ and Cl^- ion concentrations at the secretory region is of the order of 340 to 350 mM.

The results of the Swedish workers may then be taken to support the isosmotic secretion view if this is changed to the following: *The parietal secretion containing HCl has always a total concentration isosmotic with the blood.*

MODE OF STIMULATION OF THE PARIETAL CELLS

Gastric secretion occurs in two phases, psychic or nervous and chemical or hormonal depending on the origin of the stimulus to the secretory glands. The chemical is in turn divided into a gastric and an intestinal phase.

1. The Psychic or Cephalic Phase

The psychic phase as shown by the work of Pavlov and his school (1910) occurs as a result of reflex stimulation of the vagal nerve endings in the stomach. It does not occur after vagotomy in the experimental animal.

The reflexes arise from the sight or smell of food, and from various sensory stimuli in the mouth and as a result of swallowing. Since the psychic secretion has also been produced with decerebrate dogs (Lim, Ivy and McCarthy, 1925) the term "cephalic secretion" would appear preferable.

In the psychic secretion the chief or peptic cells, the parietal cells, and the mucus secreting cells are all stimulated. It may be assumed that the secretory stimulus is mediated by acetylcholine liberated at the nerve endings. Dale and Feldberg (1934) showed the liberation of acetyl-

choline in the muscular layer, but it does not appear to have been similarly demonstrated for the mucosa. Acetylcholine and its group, acetyl-*β*-methylcholine (mecholyl), carbaminoylcholine, pilocarpine, and the choline esterase inhibitors, physostigmine and prostigmine, are all effective in stimulating a flow of gastric juice.

Small doses of atropine inhibit more or less the action of these drugs or of vagal stimulation, as may be expected.

Necheles *et al.* (1938) showed that acetylcholine is very much more active in the dog and in doses as low as 50 μg. when associated with 1 mg. of prostigmine. This inhibits the choline esterase and protects acetylcholine. Otherwise relatively much larger doses must be administered.

Gray and Ivy (1937) with Pavlov pouch dogs showed that mecholyl is more active, weight for weight, than histamine acid phosphate and from 100 to 200 times more potent than acetylcholine.

With physostigmine or eserine, an esterase inhibitor like prostigmin, the two choline esters have a similar effect.

Acetylcholine and histamine. The action of acetylcholine on the parietal cells is interpreted by Babkin (1938, 1950) as being mediated by histamine, which he regards as the special stimulator of these acid-producing cells, and as being normally involved in their activation.

Babkin cites the experiments of Stavraky and of Morton (Stavraky, 1945; Morton, 1947; and Morton and Stavraky, 1948) in which acetylcholine injected into the arteries supplying the lesser curvature excited a flow of juice of low acidity but rich in mucus and pepsin. Injected into a branch of the splenic artery supplying the greater curvature an alkaline mucoid secretion was obtained (pH of 7.6 to 8.9) with low total chloride (94.5 to 144.3

m.eqs./l.) and no pepsin. Injection of histamine into the splenic artery elicited its characteristic reaction but when given along with acetylcholine a typical vagal response was found, namely, an acid juice rich in pepsin with very appreciable mucus, but somewhat lower chloride and acidity than histamine juice.

Insulin stimulation. Insulin, injected to produce a hypoglycemic condition centrally, acts as a powerful stimulant of the vagal endings in the gastric mucosa. At the same time it inhibits salivary secretion. The vagal stimulation so obtained is of greater potency and duration than that obtained by other means. (Ivy, 1930, reviews the literature.) In his extensive investigations on the human subject Ihre gave regularly 16 I.U. (Insulin "Leo") intravenously. About 40 minutes later the flow began, and the secretory impulse seemed to be associated with the lowest point of the hypoglycemia.

2. The Chemical Phase

Gastric components. Shortly after the food enters the stomach this phase begins and becomes maximal in the second hour when the psychic phase is falling steeply, and then continues for some hours.

The entry of food into the pyloric region stimulates the chemical phase, and different substances have different effects. Proteoses and peptones, as well as meat and liver extracts, produce marked stimulation.

Edkins (1906) found that an extract of the pyloric mucous membrane induced a marked secretion of gastric juice when injected subcutaneously. The active principle in the pyloric extract was termed *gastrin*, and was considered analogous to the *secretin* production of the small intestine.

This view was controverted by Zeljony and Savich

(1911 to 1912) from Pavlov's laboratory (Savich later accepted hormonal secretion, 1922). These investigators found that local painting of the pyloric mucosa with cocaine prevented the action of the usual secretagogues. Administration of atropine also prevented it. Such results were interpreted as the paralysing of the afferent and efferent side of a reflex arc.

Ivy (1941) explained the apparent significance of the cocaine painting. In a gastric mucosal transplant he showed that even though painted with procaine, it still secreted in the chemical phase of digestion. On the other hand, it did not secrete if the pyloric mucosa of the main stomach was first painted.

Popielski (1920) also attacked the view that a specific hormone was found in the pyloric mucous membrane, and attributed the injection effect of an extract thereof to a substance of unknown nature which he called *rasodilatin;* this he believed to be generally present in tissue extracts. From the work of Dale and Laidlaw (1910 to 1911; 1911 to 1912), and of Barger and Dale (1910 to 1911) Popielski considered that his *rasodilatin* might be histamine, and as a result discovered (1920) its remarkable stimulating effect on the gastric glands.

Histamine and Gastrin

Histamine stimulates gastric secretion after section and degeneration of the vagus nerves (Popielski, 1920; Keeton, Luckhardt and Koch, 1920; and Koskowski, 1922) and, as Popielski has shown, its effect is not abolished by atropine. The injection of 0.5 to 1 mg. of histamine subcutaneously into a dog or cat produces, after a short latent period of 7 to 10 minutes, a copious flow of gastric juice of high acidity. The secretion continues for about an hour and a half.

It is a curious fact that if this whole dose is injected intravenously it is ineffective. Yet minute doses injected continuously into a vein produce a continuous flow of gastric secretion (Ivy and Javois give 2.7 μg./minute/kg. for a dog as an effective dose). Teorell used continuous intravenous injection (1932, 1933) in dogs and could regulate the flow of secretion by the injection rate.

Site of action of histamine. It was shown in Babkin's laboratory (Babkin, 1930; Webster, 1931) that histamine stimulated the acid secreting cells of the gastric glands, and had no effect on the chief cells and very little effect on the mucous secreting cells. A washing out of pepsin in the first period of the secretion may simulate stimulation of the chief cells. (This may explain the observations of Bucher, Ivy and Gray, 1941.)

Bowie and Vineberg (1935) in Babkin's laboratory showed, by combined physiological and histological studies, that, whereas vagal stimulation produces marked signs of exhaustion in the chief cells and also in the mucus secreting cells, histamine stimulation produces none. Some effects on the parietal cells could be observed, but since the granules in these cells are mitochondria, they did not diminish with stimulation. It was thus established that *histamine acts as a specific stimulator of the parietal cells.*

Role of histamine in normal secretion. Ivy (1930) had suggested that histamine might in fact be the pyloric hormone exciting the chemical phase of gastric secretion, and Sacks, Ivy, Burgess and Vandolah (1932) showed that active extracts of the pylorus were no longer active as gastric excitants when subjected to the action of histaminase.

Babkin had likewise advanced the hypothesis that vagal stimulation acted through histamine in its effect on the parietal cells.

Babkin's view was based on such facts as:

(a) Histamine is the strongest of all stimulants of the parietal cells.

(b) In vagal juice, histamine is present in about the same concentration as in histamine juice.

(c) Acetylcholine, or choline when introduced from without, does not stimulate the parietal cells to the same extent as when the vagus is stimulated; though why this is so is not clear.

Whatever may be the fate of the hypothesis that the parietal cells are always finally stimulated through histamine it would appear from what follows that, with respect to the normal hormone of the pyloric region of the stomach, the wheel seems to have come full circle, with Edkin's original view confirmed.

Gastrin. Working on the protein content of the pyloric extract instead of the non-protein, Komarov (1942) in Babkin's laboratory succeeded in preparing a protein substance which is specific to the pylorus and excites on injection a flow of 1 ml. per 2 to 3 mg. It is entirely free from histamine. Uvnas (1945) appears to have obtained a more active form using a tannic acid precipitation method.

Babkin named Komarov's preparation "gastrin," adding rather meticulously that "there is no implication that it plays any part in normal gastric secretion."

Like secretin, gastrin is a protein but excites no flow of pancreatic juice, while secretin, on the other hand, excites no flow of gastric secretion.

Harper (1946) applying Mellanby's (1932) method for preparing secretin has in turn confirmed Komarov's results. Gastrin excites a copious flow of gastric juice, with a high acidity and a low pepsin content, and in this

way its effect is similar to histamine (Komarov, 1942; Uvnas, 1945; and recently Linde, 1950).

Very possibly gastrin stimulates the parietal cells by the liberation of histamine within or near them, a view first advanced by Emmelin and Kahlson (1944) and supported by Linde (1950).

Intestinal Component of the Chemical Phase

This phase arises through chemical stimulation of the intestine. Pavlov and co-workers (Babkin, 1928) showed that many substances introduced into the duodenum, separated from the stomach, excited a flow of gastric juice in dogs.

There is a long latent period of one to three hours in the gastric secretion after the introduction of foodstuffs directly into the duodenum.

The mechanism of action is unknown though probably humoral, Babkin considers the possibility of an "intestinal gastrin."

SUMMARY

The secretion from the parietal cells would appear to have the following approximate composition:

Total concentration.....340 mM. (isosmotic with blood)
H^+ ion concentration....160 mM.
Cl^- ion concentration....170 mM.
K^+ ion concentration....10 mM.
pH.....0.9

The very high primary acidities found by Teorell and by Linde, Teorell and Öbrink, using the glycine technique, can be explained by back-diffusion of the buffer and uptake of H^+ ions. At low rates of flow it would then require

double the normal secretion of HCl per unit volume of fluid to cause an outward flow if this depends on the osmotic exchange of water. Such an explanation was in part advanced by Teorell.

The isosmotic relation may then be expressed in the following form: *The parietal secretion containing the HCl of the gastric juice has always a total concentration isosmotic with the blood.*

A summary is given of present knowledge concerning the mode of stimulation of the parietal cells.

CHAPTER III

GENERAL BIOLOGICAL SECRETION OF HYDROGEN IONS

Yeast. From the facts to be described later it will appear that the secretion of H^+ ions by the gastric mucosa is only one aspect of a general biological process, and that the underlying mechanism of secretion of acid by ordinary bakers' yeast is similar to that operating in the parietal cells.

Other microorganisms besides yeast, as is well known, secrete acid. Thus *Aspergillus niger* under suitable conditions, converts glucose very largely into citric acid. Such citric fermentation by moulds was first shown by Wehmer (1893) and has developed into an industrial process in which yields of citric acid amounting to 60 to 70 per cent of the added sugar can be regularly obtained in the course of a fermentation lasting nine or ten days (an account is given by Doeleger and Prescott, 1934). Other varieties of mould, *Aspergillus fumaricus*, may form fumaric acid (Wehmer, 1918). Again, *Aspergillus niger*, by varying the conditions, will secrete gluconic acid; small amounts of oxalic, glyoxylic and other acids also appear in the external fluid (Walker, 1949, gives a full review).

Among the bacteria, conversion of sugar into lactic acid is effected by a number of bacteria, the chief being varieties of *Bacillus lactis-acidi; B. lactis aerogenes;* and *B. Delbrückii.*

An example of bacteriological production of fatty acid from sugars is that of butyric acid by *Bacillus butyricus* and related organisms, which is used in the industrial production of butyric acid. Pasteur was the first to define this type of fermentation and by inoculating glucose solution by an organism in decaying cheese could produce considerable amounts of butyric acid. Pasteur likewise demonstrated the formation of acetic acid from alcohol by an organism he termed *Mycoderma aceti*. Later investigation showed that many bacteria have this power. The most important of such organisms for acetic acid production are *B. xylinus* of A. J. Brown (1886 to 1887) commonly found in vinegar factories, and Hansen's *Bacterium aceti* found in lager breweries. In such secretion of organic acid into the external medium, there is, very often, depending on the buffering capacity of the substrate, a low pH and a concentration ratio of H^+ ions across the cell membrane of more than one thousand times.

From the studies of acid production in yeast (Conway, 1949) the general process of excretion of the organic acid may, like the secretion of succinic acid by the yeast cell, as discussed later, be divided into:

(a) Active excretion of the anion of the organic acid, formed metabolically within the cell.

(b) Addition of free H^+ ions in the cell wall, by the ionization of the reduced form of a metal catalyst, which receives H atoms from dehydrogenases, either directly or via an intermediate carrier.

In this way there is no direct concentrating of H^+ ions across the cell wall. The manner of formation of the organic anion is not significant in the present context; but it will appear that, by comparison with the HCl

secretion of the parietal cell, once formed, it is treated analogously with the Cl^- ion.

Concerning the secretion of mineral acids by the animal organism, other than gastric HCl, there is the curious example of the *Dolium galea*, (Troschel, 1854), secreting H_2SO_4 from a kind of salivary gland. Preyer (1866) described the secretion as containing 4 to 5 per cent H_2SO_4, whereas Heilbrunn (1947) gives a figure of 3 per cent. As is well known, H^+ ions are also excreted by the distal tubule in the kidney of higher animals (Smith, 1937) but in concentration and amount greatly inferior to that of secretion by the parietal cells.

COMPARISON BETWEEN THE LEVELS OF H^+ ION SECRETION BY THE YEAST CELL AND THE PARIETAL CELL

In this and subsequent chapters the acid formation by yeast will be examined in comparison with that of the HCl secretion by the gastric mucosa, and it will be seen that the underlying mechanism is the same or very similar, though whether this process in yeast may be extended to cover the various forms of biological acid secretion is a matter for further investigation. It may be said, however, that the mechanism is part of a process of fundamental biological significance (see also Pulver and Verzár, 1940 to 1941) linked with active ion transport and synthetic reductions.

The secretion of acid by the stomach considered against such background appears to be the heightening and adapting to a special end or function of an ancient property of the cell, providing another example in living organisms of economy of means to ends.

The parietal cell situated in its natural tissue environment is necessarily associated with many other cells in a

functional milieu, but the yeast cell is a free unit and the amount and mechanism of its acidic secretion can be studied uncomplicated by the action of other cells.

RATE OF ACID EXCRETION BY THE FERMENTING YEAST CELL AND THE ACTIVE PARIETAL CELL

In Table I is given the level of average secretion rate of H^+ ions from human and rat parietal cells, also from the gastric mucosa of the frog and in turn for the yeast cell.

TABLE I

ACID PRODUCTION BY THE FERMENTING YEAST CELL AND THE ACTIVE OXYNTIC OR PARIETAL CELL

Type of cell	pH reached by secretion or external fluid	Secretion rate as m.eqs./kg./hour (wet weights)
Parietal cells, human mucosa	0.9	2,500
Yeast cells (bakers' yeast) 1:250 of 5 per cent glucose (38° C.) (oxygenated)	3.2	625
Parietal cells, rat mucosa	0.9	300
Frog gastric mucosa (25° C.)	1.0–2.0	30 (72—maximum)
Yeast cells, 1:0.6 of 5 per cent glucose containing M/5 KCl (prior oxygenation) (18° C.)	1.65 (minimum of 1.4)	50 (100—maximum)

In the above table, the order of figure for the acidic secretion rate has been stated, rather than the mean as a formal statistical expression. The data for the human secretion rate are taken from Ihre (1939) with mucosal weight, and fractional volume of the parietal cells from Davies (1948). Data for the rat are from Davenport (1946) and for the frog mucosa from Davies (1948), the average secretion rate being roughly calculated from his figures. To obtain the rate from the oxyntic cells of the frog mucosa the figures above should be multiplied by a factor which is probably of the order of 2 to 4. Yeast cell data are from the author's laboratory (Conway and O'Malley, 1946; Conway and Brady, 1947; 1950, and Conway and Carton, 1950). With the concentrated suspension of yeast (1:0.6) the mean rate for the first 30 minutes has been taken.

For the frog mucosa the exact data for the proportion of oxyntic cells are not available. The pepsin of the frog's gastric juice is secreted, mainly at least by the oesophageal glands as first shown by Swiecicki, 1876, and the histology of the glandular tubules in the mucosa is different from that in the mammal as previously noted. (Vonk, 1941, reviews the subject.)

The yeast data are for bakers' yeast which was washed and suspended in 5 per cent glucose containing M/5 KCl. For the observations at 38°C., one volume of the centrifuged yeast was suspended in 250 volumes of the solution. Where the highest concentration of H^+ ion production was sought, the proportion of yeast to suspending fluid was 1:0.6, KCl being incorporated in the glucose solution to a strength of M/10 or M/5.

It follows from Table I that the figure for the secretion rate of acid by yeast (625 m.eqs./kg./hour) is about the same, or not much different from that of the mammalian parietal cell (300 to 2500 in the same units) and the conditions for the yeast excretion as recorded in Table I are not the optimum. Buffering with citrate to a pH of 5 or 6 may be expected to increase the figure (if one is to judge from similar increases with concentrated suspensions).

It will be seen also that the excretion of H^+ ions below a pH of 2.0, and which are therefore free and unbound to organic acid, is higher for the yeast cell (over 30 minutes) than the frog mucosa as a whole, and possibly but little different from the figure for the frog's oxyntic cells.

CONDITIONS LEADING TO THE APPROACH IN COMPOSITION OF UNBUFFERED FERMENTATION FLUID TO THAT OF THE PARIETAL SECRETION

Without any buffering, and without K^+ ions outside the cells, acid is excreted by the yeast (suspension 1:1 of 5

per cent glucose) in the form of succinic acid with a trace of pyruvic acid (Conway and Brady, 1948). The succinic acid is excreted at the rate (over the first 30 minutes) of 340 m.eqs. kg. dry matter hour, 45 m.eqs. succinic acid being present per litre external fluid after about 30 minutes outside. It is of interest to note here that such

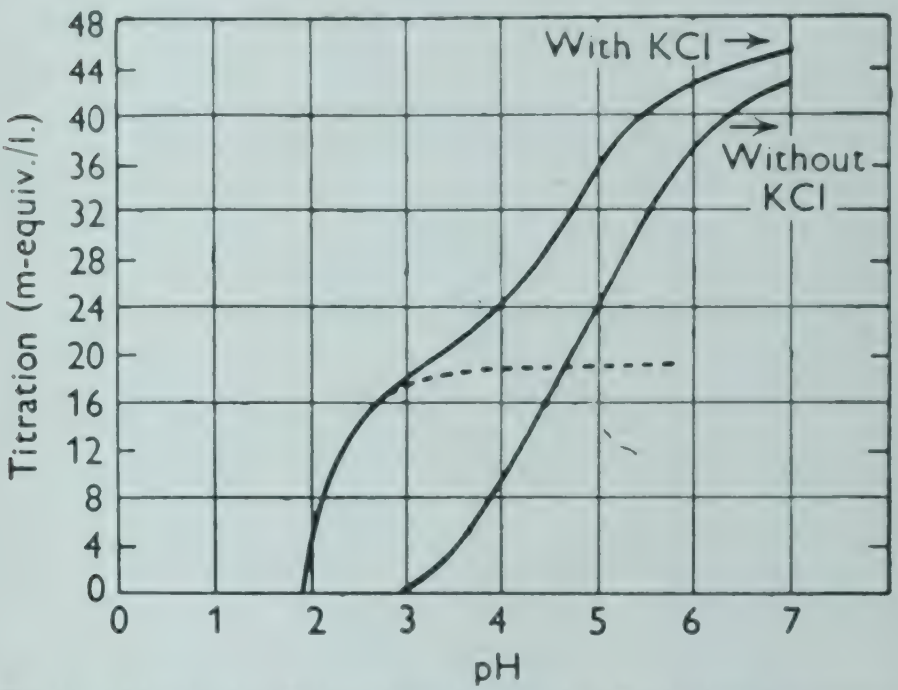

Fig. 11. Mean values for titration of suspending fluid after 30 minutes fermentation: baker's yeast at room temperature in the proportion 1 kg. washed and centrifuged yeast to 0.6 litre of 5 per cent glucose with and without N/10 KCl (Conway and Brady, 1950).
(Courtesy of *Biochem. J.*)

succinic acid is very largely present within the cells before fermentation (Brady, 1948), the succinate ions being actively excreted with H^+ ions formed as will be described later. Where the proportion of yeast to suspending fluid is small and the fermentation long sustained, considerable new formation of succinic acid from glucose

occurs, as shown by Kleinzeller (1941) and confirmed in the author's laboratory (Conway and Carton, 1950).

If in the initial 5 per cent glucose there is incorporated KCl in, say, M/5 strength there is a marked fall in the succinic acid excreted but the total acid is somewhat increased. Free HCl in quantity now appears outside the cells. The titration curves in Figure 11 show the effect

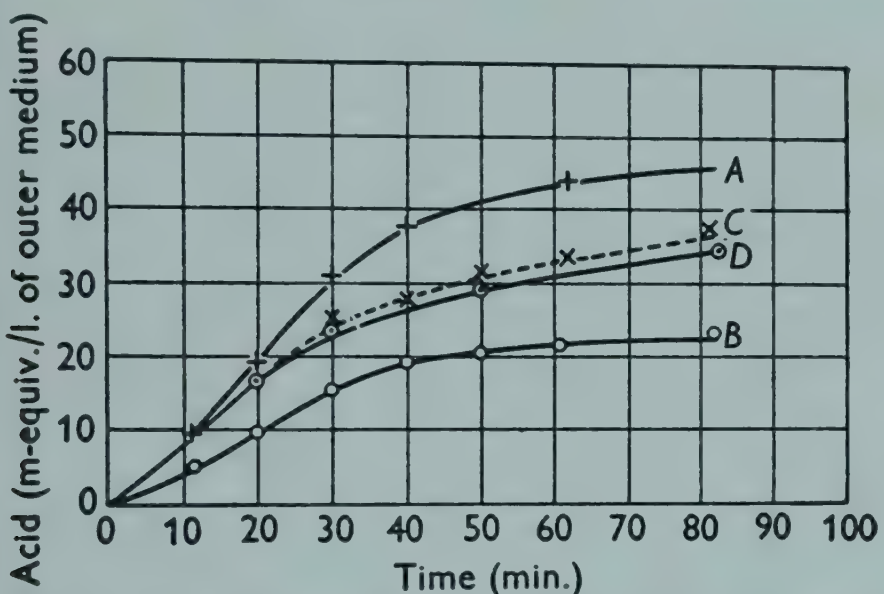

Fig. 12. Fermenting conditions as in Figure 11. Time curve of formation of total acidity (C) and of succinic acid (D) without KCl and with KCl (A and B). (Conway and Brady, 1950; Courtesy of *Biochem. J.*)

of incorporating KCl. The course of the acid secretion is shown in Figures 12, 13 and 14.

The effect was also investigated of adding KCl 30 minutes after the onset of fermentation. In this case there is a slight fall in the succinic concentration, and a rapid rise in the free acidity. The free acid arises from an exchange of K^+ for H^+ ions. This will be evident from

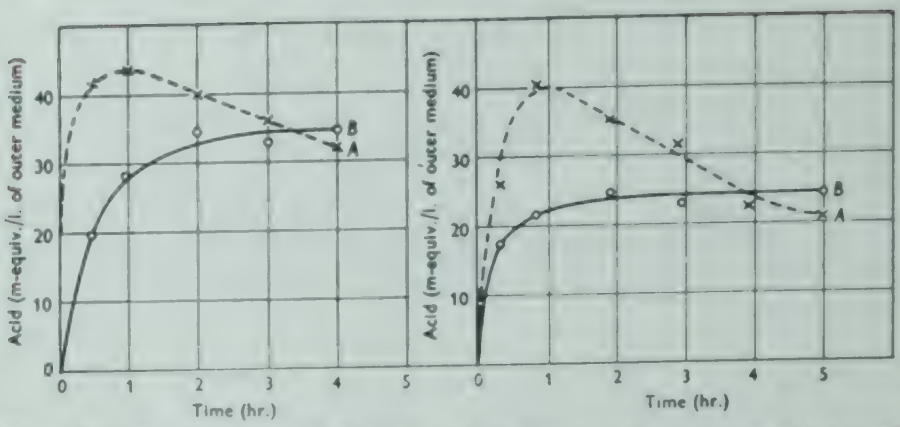

Fig. 13. Conditions as in Figure 11 but suspension one part of washed centrifuged yeast to one part of 10 per cent glucose (left-hand figure) and of 5 per cent glucose (right-hand figure), both solutions containing N/10 KCl. The curves give the total acid (upper curve) and the succinic acid in each case. (Conway and Brady, 1950; Courtesy of *Biochem. J.*)

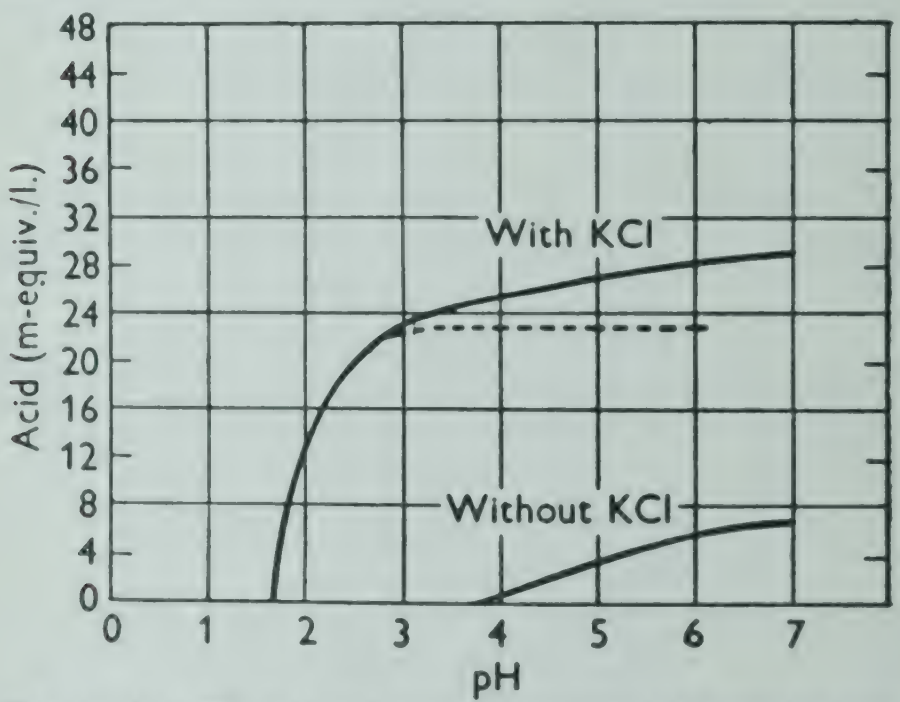

Fig. 14. As in Figure 11, with prior oxygenation of the yeast for about 30 hours. Figure 14 is to be compared with Figure 11. (Conway and Brady, 1950; Courtesy of *Biochem. J.*)

Table II (Conway and O'Malley, 1946) in which the H^+ ions appearing externally are largely balanced by an equivalent entrance of K^+ ions.

The fact that prior oxygenation tends to lessen the organic acids in yeast has long been known and utilised in experimental work. We have found that prior oxygenation extending over a few days not only greatly decreases the succinic acid in the yeast but also the amount ex-

TABLE II

K^+ AND H^+ ION EXCHANGE IN FERMENTATION

Conc. of K^+ ions in centrifuged fluid		pH of the centrifuged fluid after 20 minutes	Increase of conc. of free H^+ ions (allowing for activity correction (mM./l.)	Decrease of conc. of K^+ ions (mM./l.)
At zero time (mg./100 ml.)	After 20 minutes (mg./100 ml.)			
17	9	2.62	3	2
80	39	2.18	8	10
147	88	2.00	11.5	15
330	234	1.77	20	25
1,760	1,650	1.78	21	28

The fermenting mixture contained 1 part of yeast to 0.5 part of KCl solution plus 0.1 part of 30 per cent glucose. (Data from Conway and O'Malley, 1946.)

creted, and in an occasional experiment the succinic acid excretion may fall, practically to zero.

Figure 14 illustrates the general effect and may be compared with Figure 11 without oxygenation. As the succinic acid decreases the K^+ and H^+ ion exchange increases if KCl be present.

The Lowest External pH Levels Obtained with Yeast

The lowest pH levels are obtained with prior oxygenation. The value of 1.4 has now been observed on a few occasions and often 1.6. With progressive decrease

of the fluid volume outside the cells, the average pH approached with oxygenated yeast is 1.5 (Brady, 1949).

Without prior oxygenation and external fluid about equal to the cell volume an average pH of 1.8 is reached in 30 to 40 minutes (using 5 per cent glucose containing M/5 KCl).

TABLE III

COMPARISON BETWEEN THE YEAST SUSPENDING FLUID AND THE HISTAMINE JUICE FROM THE GASTRIC MUCOSA

	Yeast fluid	Parietal secretion of dog (Gray, 1943)	Parietal secretion of man (Fisher and Hunt, 1950)
pH	1.4 (minimum observed)	0.9	0.9
Cl^- ions	142 mmoles/l.	166 mmoles/l.	170 mmoles/l.
H^+ ions	40 mmoles/l.	159 mmoles/l.	160 mmoles/l.
K^+ ions	102 mmoles/l.	7 mmoles/l.	10 mmoles/l.
Na^+ ions	0 mmole/l.	0 mmole/l.	0 mmole/l.
Organic acid anions	2 mmoles/l. (minimum observed)	None	None

Approach in Composition of the Yeast Suspending Fluid to Histamine Juice from the Gastric Mucosa

With yeast oxygenated for some days and then fermented with 0.6 vol. of 5 per cent glucose containing M/5 KCl, the maximum H^+ ion content of 40 mM./l. was reached in the suspending fluid. Under these conditions the composition may be compared with that for the parietal cell secretion from the dog as given by Gray (1943) and for the human subject as given by Fisher and Hunt (1950) in a study of Ihre's data (1939).

Table III illustrates clearly the general similarity. The presence of K^+ ions as the only mineral ions in the parietal secretion is of interest; also the very low organic acid in the yeast fluid and its absence in the parietal secretion.

SUMMARY

The secretion of HCl by the parietal or oxyntic cell of the gastric mucosa cannot be considered a unique occurrence. Apart from the remarkably high secretion of sulphuric acid by a gland of the *Dolium galea* (and which reaches nearly 0.7 to 1.0N concentration) there occurs a striking parallelism between the acid secretion by yeast, under certain conditions, and that elicited by histamine stimulation of the gastric mucosa.

Thus the yeast cell during fermentation can reduce the external pH to as low as 1.5 in 30 minutes, when the suspending fluid is reduced to the lowest levels and the yeast oxygenated beforehand. With a suspension of 1 part of such yeast to 0.6 part of a suspending fluid containing 5 per cent glucose and M/5 KCl, the pH outside the cell reaches about 1.65 though 1.4 has been occasionally observed.

These low pH values result from a K^+ and H^+ exchange, and the fluid contains free HCl in strength corresponding to the pH level.

Although the most striking free acidity results with yeast in such K^+ and H^+ exchange, it is likely that the true analogy between the parietal secretion and the secretion of acid by yeast lies between HCl in the one and succinic acid in the other, the corresponding anions existing already in the cells.

The excretion of fixed acid by yeast is an example of a rather widespread power of organic acid secretion by microorganisms. In this one may regard the secretion of H^+ ions as independent but simultaneous with the secretion of the anions, the total resulting in the particular form of acid excreted. The HCl secretion by the oxyntic cells may be similarly viewed, as later described.

It may be added that during short period fermentation the succinic acid excreted derives from succinate ions present beforehand in the cells. In long period fermentation it arises largely from new formation of succinic acid. This restores the H^+ ions removed in the redox cycle (Chapters V and VI). A similar restoration of H^+ ions in the parietal cell is provided by carbonic acid, the anions of which exchange for Cl^- ions (Chapter X) dealt with in turn like the succinate ions in yeast.

Throughout the following chapters a fruitful comparison between the mechanism in yeast and the oxyntic cell is drawn.

CHAPTER IV

THE IMMEDIATE SOURCE OF THE HYDROGEN IONS: THE ORGANIC ACID THEORY

From the account in the previous chapter, it will be seen that the secretion of free H^+ ions in high concentration by the gastric parietal cell is not a biologically unique process. Under certain conditions it can be simulated by the yeast cell, and the fuller significance of this analogy will become more evident in later chapters. What occurs in the parietal cell leading to the formation of acid is but a special manifestation of a wide biological endowment, linked to the active transport of ions and biological reductions and syntheses.

It will therefore appear fruitful in the elucidation of the various aspects of the problem of gastric acid production to keep the yeast analogy under parallel consideration, being careful at the same time to distinguish differences when such arise.

THE REQUIREMENT OF AN OUTER ACIDIC REGION WITH MEMBRANE BARRIER TO THE INTERIOR OF THE CELL

When a pH as low as 1.4 appears outside the yeast cell resulting from a K^+ and H^+ ion exchange in fermentation, if the H ions come *directly* from the H^+ ions within the cell they move across a concentration difference of 100,000

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times and in the case of the parietal cells, it is likely that the difference is much more.

The great improbability of such a process is obvious, and some other than a *direct* source from the free H^+ ions within the cell arises for consideration.

It would appear that a probable source would be the H^+ ions formed by an ionizing organic acid (Conway and O'Malley, 1942-1946), avoiding in this way diffusion limitations, or p.d.'s of the order of 300-400 mV. across the membrane (Conway and Brady, 1947). If, however, the pH of the cell as a whole is examined (Conway and Downey, 1948, 1950) an increase in acidity does not result, but rather a *decrease*. Hence, if an ionizing organic acid be considered as the *immediate* source of the H^+ ions exchanging for K^+ ions this offers little or no apparent advantage over the direct secretion of H^+ ions from the cells, *unless an outer region of the cell is considered in which the organic acid is formed* (Conway and O'Malley, 1946) yielding high H^+ ion concentration.

Such acid must also be prevented from diffusing freely backwards into the cell, and a membrane barrier would appear essential. The same requirement holds for the prevention of the back diffusion of HCl in the parietal cell, at the region where H^+ ions first appear in high concentration along with Cl^- ions. No constraint of potential gradients or differences would suffice to prevent the back diffusion of HCl, since the electrical constraint against the cation in free solution will be the equal and opposite for the anion.

Theoretically, back diffusion might, in greater or lesser degree, be prevented by an outward flow of fluid, as considered by Franck and Mayer (1947) in their "osmotic diffusion pump." This could not apply to the yeast cell, and for the parietal cell the level of flow would be much too

high to prevent relatively large decreases in efficiency. With the parietal cell, also, conditions can be obtained (Hollander, 1949) in which the secretion retains an almost isosmotic HCl concentration, even at the lowest rates. The glycine experiments of Teorell and collaborators, discussed in the previous chapter, are here also of interest.

EVIDENCE FOR AN OUTER ACIDIC REGION IN THE YEAST CELL

If one considers centrifuged yeast, the theoretical interspace between the cells, assuming the closest packing, is 26 per cent of the whole volume (Conway and Downey, 1950). If samples of such yeast are suspended in equal volumes of solutions containing different substances, a number of such (inulin, peptone, gelatin, etc.) appear to enter a space which is 22 to 24 per cent of the centrifuged yeast volume, and which may be considered to represent the true interspace volume in centrifuged yeast. Another group which includes non-fermentable sugars such as arabinose and galactose, as well as electrolytes such as NaCl and a number of amino acids, enter very rapidly into a space which represents 31 to 33 per cent of the whole volume and do not continue to enter the yeast cell at an appreciable rate. The increase over the theoretical interspace or over that actually found by the use of inulin and peptone cannot be accounted for by adsorption, since, as with the non-fermentable sugars, it is quite uninfluenced by concentration (from 1 to 20 per cent), provided allowance is made in the calculation for the water abstracted osmotically from the cells. Figure 15 shows the water space (R) in the yeast cell, into which the external solute has entered, plotted against concentration

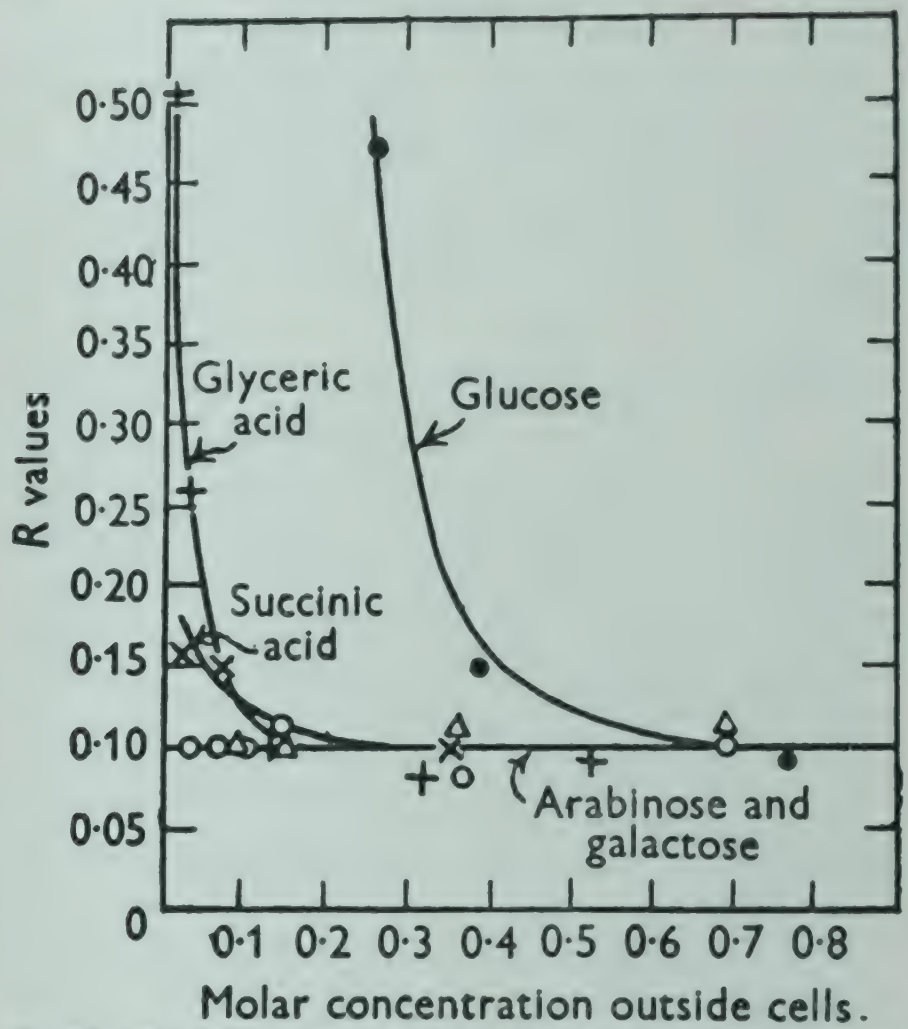

Fig. 15. The "R" value gives the amount of substance which has entered the yeast cell as a ratio of the external concentration at the time of observation (15 mins. after suspending). One kg. washed centrifuged yeast was suspended in 1 litre of solution. Inulin, peptone and gelatin give zero "R" value. (Conway and Downey, 1950: Courtesy of *Biochem. J.*)

(allowing for cell water abstracted from the cell). Besides arabinose and galactose, glucose is also represented.

The high apparent value with low glucose concentrations arises from the disappearance of the sugar in fer-

mentation. As the concentration outside increases this disappearance over short periods, such as 15 minutes, is relatively small and the space approaches the general

Fig. 16. Parietal cell from deeper part of human peptic gland (Plenk, 1932—after Lehner). Fixed in formol-alcohol and stained with Bensley's copper-chrome-hematoxylin.

figure for the non-fermentable sugars. Succinic and glyceric acids are also represented in Figure 15. At low concentrations these give high apparent "spaces," which

may be explained by some degree of buffering existing in the outer region.

The space so determined is approximately 14 per cent by volume of the yeast *cell* and since this volume is unaffected by the osmotic concentration outside, its membrane would appear to have the same distensibility as the cell wall, which offers considerable resistance to distension. The conclusion may be drawn that the region in question is the cell wall, with a water content therein approximately 0.13 to 0.14 of the whole cell volume or about 19 per cent of the total cell water.

In this outer region metabolic processes may be shown to occur such, for example, as the first stage or stages in the metabolism of glucose and the appearance there of free succinic acid. Whether these in fact occur on the surface of the inner membrane or more diffusely distributed throughout the wall is immaterial for present considerations. (This agrees with recent investigations of Rothstein, 1950, on uranium inhibition of the first stages of glucose assimilation, which he locates in the surface of the yeast cell.)

In Figure 16 parietal cells from the human peptic glands are shown (after Plenk, 1932). The intracellular canaliculi are very evident and appear to possess a distinct wall which may be considered analogous to the outer metabolic region of the yeast cell.

ACIDIFICATION OF THE OUTER REGION AND ALKALINISATION OF THE INNER DURING FERMENTATION

During fermentation the outer region becomes acid. This follows from the high concentration of free succinic acid appearing outside the cells (when fermenting in a

1:1 of 5 per cent glucose) and the free permeability to succinic acid of the outermost cell surface.

While the pH of the outer region falls that of the inner region rises, as shown by a marked overall increase of pH of the yeast cell during fermentation.

ORGANIC ACID AS THE H^+ ION DONOR

On finding that the yeast cell during fermentation could quite readily produce pH-levels in the external fluid in the region 1.6 to 1.8 and very occasionally as low as 1.4, the fermentation mixture consisting then of 1 part yeast to 0.6 part of 5 per cent glucose containing 0.1 mole or more of KCl per litre, it was considered that the H^+ ion donor might well be one or other of the acids produced in the metabolism of the glucose. Pyruvic acid or phosphopyruvic acid seemed specially indicated because of the low pK value, and it was found that pyruvic acid in small concentrations in the external fluid followed in a parallel way the production of the high acidity (Conway and O'Malley, 1946).

Concerning the application to the oxyntic cells in the stomach, it may be noted that the idea of an organic acid supplying the H^+ ions of the gastric juice was originated by Maly (1874), 50 years after Prout's discovery (1824) of hydrochloric acid therein. Maly considered the possibility of lactic acid reacting with NaCl in the lumen of the stomach, a view having now only historical interest, and abandoned later by him. The organic acid hypothesis is advanced here from a quite different standpoint.

It was thought that an acid such as phosphopyruvic acid might be formed in an acidic region of the parietal cell, similar to the acidic region in yeast, and there exchange its H^+ ions for K^+ or Na^+ ions derived from a saline secre-

TABLE IV

CYCLICAL SCHEME FOR THE PRODUCTION OF HYDROCHLORIC ACID

	Lumen	Intracellular substance	Blood or extracellular fluid
1.		$R{\cdot}CH(OH)_2 + B + Cl^- + Ac$	$Cl^- + CO_2 + H_2O$
2.	$B^+ + Cl^-$	$R{\cdot}CH(OH)_2 + Ac$	$Cl^- + CO_2 + H_2O$
3.	$B^+ + Cl^-$	$R{\cdot}COOH + AcH_2$	$Cl^- + CO_2 + H_2O$
4.	$B^+ + Cl^-$	$H^+ + R{\cdot}COO^- + AcH_2$	$Cl^- + CO_2 + H_2O$
5.	$H^+ + Cl^-$	$B^+ + R{\cdot}COO^- + AcH_2$	$Cl^- + CO_2 + H_2O$
6.	$H^+ + Cl^-$	.....	Cl^-
7.	$H^+ + Cl^-$	$R{\cdot}COOH + B^+ + HCO_3^- + AcH_2$ $R{\cdot}CH(OH)_2 + B^+ + Cl^- + Ac$	HCO_3^-

tion. The alkali salt of the acid would then return to the inner region of the cell. The subsequent fate of the organic acid seemed at this stage unessential, but it was regarded as oxidized with a resulting alkalinity equivalent to the acid secreted. A series of investigations of human gastric acid secretion was undertaken to investigate the possible association of pyruvic acid with the production of free H^+ ions (Conway, FitzGerald and Walls, 1945). The investigation, however, did not result in anything definite.

A somewhat similar theory as to the origin of the H^+ ions in the parietal secretion was advanced about the same time by Bull and Gray (1945). In their hypothesis a solution containing organic acid was secreted into the canaliculi of the parietal cell and at some region had its organic acid anion exchanged for Cl^- ions thus producing HCl in the fluid passing to the lumen of the gland.

Somewhat later the finding by Davies (1948) that in the

isolated gastric mucosa of the frog the molar production of HCl could exceed the molar consumption of oxygen, made it probable that, if an organic acid operated in such a way, it was cyclically reformed (Conway, 1947). Otherwise the maximum value of the ratio Q_{HCl}/Q_{O_2} would be only 1.0, provided it was established that the carbohydrate utilised was entirely oxidized, and that no other changes significant for H^+ ion production occurred. The views of Davies *et al.* on this subject are considered further in Chapter VI.

A scheme of the following kind was then put forward to illustrate how the cyclically re-formed organic acid donor might operate (Conway, 1944).

In this scheme illustrated in Table IV, inorganic chloride ($B^+ + Cl^-$) in about isotonic strength is secreted into the lumen; glyceraldehyde then yields H atoms to an acceptor and the resulting acid exchanges H^+ ions for B^+ ions, HCl appearing in the lumen. The reversal of the oxidation process then occurs in another cell region and HCO_3^- ions interchange for Cl^- ions. The overall reaction may be written:

$$CO_2 + H_2O \rightarrow H_2CO_3 \rightarrow H^+ + HCO_3^- \qquad (1)$$

This, however, obscures the fact that in the scheme the *immediate* source of the H^+ ions is glyceric (or phosphoglyceric) acid. It may also be pointed out that the glyceraldehyde-glyceric acid system was used for illustration only.

While the occurrence of such a cyclical process was not assumed for yeast, or at least was not necessary to explain the facts, it was thought that a search for the H^+ ion donor might throw light on the nature of the acid involved in the gastric secretion. The following pages give an account of the results of this investigation.

THE SEARCH FOR AN ORGANIC ACID AS H^+ ION DONOR IN YEAST

1. The Organic Acid H^+ Ion Donor as a Stable or Non-metabolised Acid

If a relatively stable organic acid, such as succinic acid, is formed in the outer region and H^+ ions are exchanged there for K^+ ions, the organic acid anions being retained, this would at first appear to offer the simplest explanation for the formation of a high external acidity.

Permeability requirements. In the outer region then, besides the K^+ and organic acid ions, there will be relatively high concentrations of H^+ and Cl^- ions. Since the chemical potential of HCl in this outer region will be enormously greater than that within the cells, the permeability of the inner membrane must be such as to block the simultaneous passage into the cell of both H^+ and Cl^- ions. This could be effected either by a high degree of cation or, alternatively, of anion impermeability. Here, the word impermeability is used as a convenient term to signify entrance rates that are small compared with the passage of other ions under consideration.

In yeast it is mainly achieved by passive anion impermeability of the inner membrane. At the same time, since K^+ and H^+ ions could exchange across the inner membrane, and as this occurs only very slowly (as distinct from rapid K^+ and H^+ exchanged in the outer region), the cation permeability must in turn be rather low. It may also be noted that the entrance of a H^+ ion will depend on the exit of the much larger K^+ ion, and no matter how fast H^+ ions *per se* can traverse the membrane a net change of the free acidity will turn on the rate of exit of K^+ ions from the cell.

In turn the back diffusion of the free organic acid should be comparatively slow.

Concerning the permeability of the outer surface of the cell wall, it would seem necessary that the organic acid anions be prevented from diffusing freely outward, since otherwise they will remove any appreciable concentration of free H^+ ions by the formation once more of free organic acid outside the cells.

Succinic acid as the H^+ ion donor. It is curious how much, on an initial examination, succinic acid fulfils the various conditions. Thus when yeast ferments 5

TABLE V

RETENTION OF SUCCINIC ACID IN THE YEAST CELLS IN THE PRESENCE OF KCl

Nature of suspension (1 part of moist yeast to 0.6 part suspending fluid)	Succinic acid in the suspension (mg. per cent)	Succinic acid in the fluid after centrifuging (mg. per cent)	Succinic acid in the yeast cells (calculated value) (mg. per cent)	pH of suspension
Without KCl	85	155	12	3.03
With KCl	78	111	44	1.97

per cent glucose in a 1:1 relation, unbuffered and without KCl, a considerable amount of organic acid is excreted, which is almost entirely succinic acid. (There is likewise a small amount of pyruvic acid of the order of 1 m.eq./l., and this can increase to about 8 m.eqs./l. when the pH is buffered externally to about 5 to 6, Conway and Winder, 1950.)

When the fermentation occurs under the same conditions with M/10 KCl included in the 5 per cent glucose, the total titratable acidity is not much increased, and the succinic acid falls markedly, being replaced by hydrochloric acid. This is shown in Figure 11, Chapter III.

Corresponding to the fall in succinic acid excreted there is a retention of succinate within the cells.

Table V gives the results of three sets of experiments illustrating this point.

It will appear that succinate is retained in the cells with absorbed K^+ ions, when KCl is present during fermentation.

Furthermore the inner membrane of the cell wall shows a high degree of impermeability to free succinic acid, and succinate ions do not appear to traverse freely the outermost cell surface.

Such correspondences are very striking, but even if succinic acid were to act as a direct donor of H^+ ions only the first ionizing COOH group could be considered as yielding H^+ ions with the comparatively low pH in the outer region, and the retention of succinic acid in the cell would at most account for only 50 per cent of the free H^+ ions formed in the K^+ and H^+ ion exchange.

Facts Leading to the Rejection of the Hypothesis of Succinic Acid or any Similar Non-metabolised Acid as a Direct Donor of H^+ ions, in the K^+ and H^+ ion Exchange.

(a) The succinic acid excreted under the conditions described above has been very largely present within the cells as succinate ions. Thus the total succinic acid of the suspensions increases very little and there seems for the most part only a shift of the succinate system from within the cell to the external solution.

So far from the succinate system being a direct donor of H^+ ions, it must be itself an acceptor of H^+ ions from some other source, free succinic acid being formed.

(b) If the yeast is oxygenated prior to fermentation there is a marked decrease in the succinic acid excreted and in the total succinic content of the suspensions. This may proceed to the almost complete disappearance

of succinic acid from the suspensions or the external fluid both before and after fermentation, and no other organic acid is substituted for succinic acid. *Yet under such conditions the maximum free acid is formed by the exchange of K^+ ions and H^+ ions (see Table VI).*

(c) Apart from succinic acid and a very small amount of pyruvic acid no other organic acids appear in the external fluid outside the yeast cell and no appreciable amount of ether-extractable acids increases in the cells

TABLE VI

THE BEHAVIOR OF SUCCINIC ACID DURING FERMENTATION AFTER PROLONGED OXYGENATION

Time of fermentation (minutes)	pH of suspension	Suspending fluid		Yeast cells	
		Succinic acid (m.eqs./l.)	Total ether-extractable acids (m.eqs./l.)	Succinic acid (m.eqs./l.)	Total ether-extractable acids (m.eqs./l.)
0	...	0.0	1.0	1.4	13.3
24	1.53	0.0	2.9	4.6	15.2

The succinic acid concentrations are expressed with reference to one liter of the suspending fluid or outer medium. This experiment shows the practical disappearance of succinic acid from the suspending fluid (0.6 pts. of 5% glucose with M/5 KCl to 1 pt. yeast) after prolonged oxygenation, and yet the maximum free acid is reached.

during fermentation after prolonged oxygenation under the conditions described.

Besides such evidence direct analyses of citric, isocitric, cis-aconitic, phosphopyruvic and total keto acids, as well as acetic and other volatile fatty acids, give either very small concentrations or negative results.

2. The Organic Acid H^+ Ion Donor as a Metabolised Acid

From the previous account it will be seen that no evidence of a non-metabolised acid acting appreciably as a H^+ ion donor could be obtained. Without prior oxygena-

tion succinic acid may play this role to a relatively small extent, but for the most part the succinate system is not a donor but an acceptor of H^+ ions under the conditions examined. The question then arises, if a metabolised acid supplies them.

During fermentation a large amount of acid other than carbonic is formed.

In the fermentation of glucose by baker's yeast, about 70 per cent of the reaction

$$C_6H_{12}O_6 \rightarrow 2CO_2 + 2C_2H_5OH \qquad (2)$$

is realized. With the yeast fermenting 5 per cent glucose (1 part of centrifuged yeast, or 0.8 part of yeast cells, to 0.6 part of 5 per cent glucose) and assuming that the fermentation leading to CO_2 passes through the stage of glyceric acid, then at such a stage there is a total formation of approx. 290 mM. of H^+ ions per kg. of yeast cells. This large amount of acid disappears in one or other of the following reactions:

(a) $$R\cdot CO\cdot COO^- + H^+ \rightarrow R\cdot CO\cdot COOH \rightarrow R\cdot CHO + H_2CO_3 \rightarrow R\cdot CHO + H^+ + HCO_3^- \rightarrow R\cdot CHO + CO_2 + H_2O \qquad (3)$$

(b) $$R\cdot CO\cdot COO^- + H^+ \rightarrow R\cdot CO\cdot COOH \rightarrow R\cdot CHO + CO_2 \qquad (4)$$

(c) $$R\cdot CO\cdot COO^- + H^+ + H_2O \rightarrow R\cdot CHO + HCO_3^- + H^+ \rightarrow R\cdot CHO + CO_2 + H_2O \qquad (5)$$

In (a) and (c) HCO_3^- ions are formed directly (via H_2CO_3 in (a)). For these two paths it is considered that CO_2 is not directly formed by decarboxylation, in (b) it is formed directly. Evidence for direct formation of CO_2 in decarboxylation has been somewhat conflicting (Conway and O'Malley, 1948; Krebs and Roughton, 1948) and it would appear to depend upon the total conditions (Conway and O'Malley, 1951).

Though a great amount of acid other than carbonic is thus formed, yet in the processes of decarboxylation and release of carbon dioxide it is all used up again. As a method of providing H^+ ions for excretion, it offers no advantage over the free H^+ ions of the cell itself, *when the ionization and decarboxylation processes proceed in the same region or chamber.*

But if the decarboxylation process takes place in the inner region and the initial stages leading to pyruvic acid, or pyruvic acid itself, appear in the outer region, there could arise a suitable release of H^+ ions. Such a view may be rejected for the following reasons.

REASONS FOR REJECTING THE HYPOTHESIS OF A METABOLISED ORGANIC ACID AS H^+ ION DONOR FOR YEAST ACIDITY

(a) The question arises, when H^+ ions in the acidic region exchange for K^+ ions, how do the anions of the H^+ ion donor enter the main region and become metabolised? Either they exchange for other anions, which are not metabolised (otherwise they would take up H^+ ions) or they pass with K^+ ions across the inner membrane.

The first of these hypotheses introduces again a non-metabolised acid, differing from succinic, present in large amounts, and which certainly does not appear in the external fluid. Both hypotheses require a rapid diffusion inwards of K^+ ions as well as the organic acid anions, which would make it very difficult to understand why H^+ and Cl^- ions do not diffuse back with similar rapidity and prevent any appreciable accumulation of HCl outside the cell.

(b) Also the excretion of succinic acid in the absence of KCl would require to be explained by an exchange of

the anions of succinic acid for the anions of the organic acid involved, the H^+ ions of the latter joining to succinate to form succinic acid. Here again there arises a rapid exchange of anions across a membrane which otherwise appears practically impermeable to anions.

CARBONIC ACID AS A DIRECT DONOR OF H^+ IONS

This question arises because in the exchange of K^+ for H^+ ions in yeast a production of bicarbonate to upwards of 70 to 80 per cent equivalence of the H^+ ions excreted can be observed within the cell, and when at the same time there is no appreciable increase of ether-extractable acids.

(To demonstrate the large bicarbonate formation it is necessary to maintain the carbon dioxide tension at atmospheric pressure.) Further there is evidence to show that when pyruvic acid is decarboxylated carbonic acid is directly formed (Conway and O'Malley, 1948). Whether free CO_2 or carbonic acid is directly formed appears to depend on the conditions (as noted above, Conway and O'Malley, 1951).

Assuming that the process goes through HCO_3^- ions or H_2CO_3 molecules and not directly through CO_2 it will be seen from equations (a) and (c) above that the H^+ ions appearing with HCO_3^- ions merely maintain the H^+ ion arising from the formation of glyceric acid and continued down through pyruvic and finally carbonic acid. If at this last stage H^+ ions are exchanged for K^+ ions in the outer region K^+ and HCO_3^- ions would then accumulate therein and in a concentration of about 40 mM. 1. The carbonic acid hypothesis then becomes untenable chiefly for the reason that with free diffusion of small ions

to and from the outer region and the external solution all the free H^+ ions would very rapidly be taken up by HCO_3^- ions.

To avoid this it would be necessary to suppose that the HCO_3^- ion exchanges with an anion from within the cell or that it diffuses quickly into the cell with a K^+ ion. The difficulty of considering similar exchanges has been discussed above.

Although the facts lead to a rejection of carbonic acid as a direct donor of H^+ ions, yet the carbonic acid system would appear to play a very significant role as an indirect donor maintaining the pH of the cell. For the cyclical organic acid scheme given above for gastric acid secretion the overall equation may be written:

$$CO_2 + H_2O \rightarrow H_2CO_3 \rightarrow H^+ + HCO_3^- \qquad (6)$$

the HCO_3^- ions subsequently exchanging for Cl^- ions, and this applies also for the "redox" theory discussed below.

In this sense then Davenport's view (1943) of the significance of this system as yielding H^+ ions in gastric secretion is still valid and is discussed in Chapter X.

SUMMARY

The concentration difference of H^+ ions across the cell wall or membrane in yeast or parietal cells, when secreting H^+ ions at maximum concentration, is so great that no direct concentrating of the cellular H^+ ions can be considered as occurring. An organic acid is probably the direct donor, and for the gastric mucosa, the high value of Q_{HCl}/Q_{O_2} found by Davies for the isolated frog's mucosa makes a cyclical hypothesis most likely for the operation of the organic acid involved.

A search for a *direct* organic acid donor in yeast, and of glucose metabolite type has not been successful and shows that under the conditions studied, neither succinic acid nor carbonic acid can play this role to any extent, *though quantitatively they account for the anions associated with absorbed K^+ ions, in short period fermentation.*

The evidence shows that a non-metabolised acid other than succinic acid is not involved, and there are serious permeability difficulties in the way of interpreting the H^+ ion donor as a metabolised acid.

It is shown in the following chapter that such difficulties can be resolved when the organic acid donor is considered not as a glucose metabolite but as one of the respiratory catalysts of the cell.

CHAPTER V

THE REDOX THEORY

ORGANIC ACID DONOR AS REDUCED RESPIRATORY CATALYST

THE INTRODUCTION of this view for gastric acid secretion (Conway and Brady, 1948) largely eliminated the difficulties pointed out in the previous chapter with a metabolite of glucose acting as the organic acid donor of H^+ ions. Here, the organic acid is considered to be the reduced form of a heavy metal catalyst with the H^+ ions *derived from metabolic H atoms.* The catalyst operates across the membrane in a redox cycle.

In the metabolism of glucose 24 H atoms pass to oxygen, 12 from the original carbohydrate molecule and 12 from water incorporated at various stages. The metabolic H atoms can be regarded as converted at some stage or stages, entirely into electrons and H^+ ions.

The use of such splitting of the metabolic H atoms for the secretion of the H^+ ions of the gastric juice is here briefly presented at the outset under five headings.

1. The reduction phase of the metal catalyst, operating in the outer acidic region.
2. The oxidation stage of the metal catalyst operating centrally in the cell.
3. The outward passage of Cl^- ions from a potential difference accompanying the formation of H^+ ions or possibly actively carried like succinate ions from the ast cell.

4. The restoration to the cell of H^+ ions removed in the oxidation stage.

5. The secretion of water as a sequel to the accumulation of H^+ and Cl^- ions, the secretion being thus isosmotic or nearly isosmotic with the blood.

1. Reduction of the Metal Catalyst

One may write:

$$CtH_2 + 2M^{n+} \rightarrow Ct + 2M^{(n-1)+} + 2H^+ \qquad (1)$$

in which the complex containing the metal catalyst contains only one metal atom, though it may contain more than one.

When the electrons pass to oxygen, H^+ ions are in turn withdrawn from the cell. Ordinarily the addition and withdrawal quantitatively balance without alteration of pH from such exchanges, but it may happen that the two stages are effectively separated in the parietal cell and in yeast. The acceptance of H atoms and conversion to H^+ ions and electrons would occur then in the acidic region. The electrons could be regarded as passing into the interior of the cell in an overall exchange for Cl^- ions, in the case of the parietal cell, or in yeast in exchange for succinate ions (by the possible operation of a carrier system) or alternatively accompanied by K^+ ions, in what may be termed an electro-adsorption complex.

2. The Oxidation of the Metal Catalyst

Within the cell the electrons are joined with H^+ ions and transferred to an acceptor:

$$2M^{(n-1)+} + R_1 + 2H^+ \rightarrow 2M^{n+} + R_1H_2 \qquad (2)$$

or with oxygen as acceptor;

$$4M^{(n-1)+} + O_2 + 4H^+ \rightarrow 2H_2O + 4M^{n+} \qquad (3)$$

In this latter case the oxygen is considered as reduced to water, without passing through the intermediate stage of H_2O_2. Occasionally the process is represented as a reduction of oxygen to OH^- ions. This is again referred to in Chapter VII. But however it may be presented H^+ ions are removed in amount equivalent to their formation at the reduction stage of the metal catalyst.

3. The Outward Passage of Anions

The overall exchange here is of an anion for an electron. The detailed mechanism of that transfer is at present a matter for speculation. The outward passage of the anions may result simply from a potential difference developing along with the accumulation of H^+ ions; or they may be actively carried in the return of the oxidised catalyst to the acidic region or to its operation therein.

In yeast also nearly all the anion transfer or passage can be substituted by an inward movement or carriage of K^+ ions. Thus, a K^+ ion in an overall transfer enters with an electron. But though the lowest pH levels are then obtained with yeast, the true parallel may lie between the secretion of succinic acid by yeast and of HCl by the parietal cell. Here it may be said that the acidity of the succinic acid excretion behaves in every way examined like the H^+ ions exchanged with K^+ ions. It is of special interest that, using a pure glucose solution for the yeast suspension, the resulting almost pure succinic acid excreted (in a 1:1 suspension) contains also K^+ ions at the same level as the pure parietal secretion.

4. The Restoration of H^+ Ions to the Cell

With such a process going on the cell would become more and more alkaline. This is prevented in the case of the parietal cell by the reaction:

$$CO_2 + H_2O \rightarrow H_2CO_3 \rightarrow H^+ + HCO_3^- \qquad (4)$$

with subsequent interchange of Cl^- ions for HCO_3^- ions (Davenport, 1943, 1946).

For yeast over long period fermentations, or where the amount of yeast is small compared with the amount of glucose containing suspending fluid, the alkalinity in the cell is balanced by a new formation of succinic acid, with excretion of its anions, which are in turn associated in the outer region with H^+ ions from the metal catalyst and appear as free succinic acid in an unbuffered medium.

5. The Parietal Secretion of Water Containing H^+ and Cl^- Ions as a Sequel to the Accumulation of these Ions

The view that the parietal secretion is isosmotic with the blood is well supported by the evidence and has been discussed in Chapter II. It may be interpreted here as arising from the formation of slightly hypertonic concentrations of H^+ and Cl^- ions in the acidic region. Water is then abstracted osmotically from the cell, and so from the blood. In this way it would appear that the parietal secretion must always be close to an isotonic secretion of HCl (including, from the evidence, some small amount of K^+ ions). When slowly secreted it becomes reduced in concentration in the lumen or gastric cavity by various factors or in varying degrees as discussed in Chapter X.

The introduction of the redox theory for gastric acid secretion (Conway and Brady, 1948) was quickly followed by a somewhat similar treatment of the origin of gastric acidity by Crane and Davies (1948). Patterson and Stetten (1949) also proposed a theory based on the operation of a redox catalyst; and recently Rehm (1950) has expressed similar views. These are briefly discussed later.

The concept of a redox cycle acting at a surface was used by Lund and Kenyon (1924, 1928) to explain the source of bioelectric potentials in plant roots. Their interpretation has been criticised for involving the passage of electrons through water media, and in model experiments at least a metallic medium is essential. Similar criticism has been advanced against Stiehler and Flexner's interpretation (Stiehler and Flexner, 1938) of the observed opposite movements of basic and acidic dyes in the choroid plexus secreting cerebro-spinal fluid, and referred to a current established by reversible redox systems.

Such views refer to the actual passage of current arising from differences of redox potentials. Lundegårdh (review given in 1947) used the idea of the redox cycle of a catalyst (ferricytochrome) as a non-specific carrier of anions into root hairs. The loss of an electron on oxidation would create a surface condition favourable for anion carriage.

Robertson, developing Lundegårdh's views, has also made recent contributions of interest (Robertson and Wilkins, 1948a, 1948b) concerning such active transport of anions into root hairs (further noted in Chapter VI).

EVIDENCE FOR THE "REDOX" THEORY

Such evidence already considered for the cyclical formation of an organic acid likewise applies, when the organic acid is a reduced metal catalyst.

It will be seen that the cyclical formation of organic acid in an acid-aldehyde sequence as previously exemplified is also a "redox" theory, but here the development so termed is concerned with the process as regards the catalysts rather than metabolites, though as is well known, the distinction is sometimes difficult to draw.

But with a system of redox catalysts the cyclical operation is a necessary feature of their activity, and thus there is an economy of assumptions.

The "redox" theory outlined above considerably lessens the permeability difficulties associated with the concept of the immediate origin of H^+ ions from an organic acid subsequently metabolised.

Thus, when it is considered that in the case of the yeast cell K^+ ions enter the inner region with electrons, or by specific adsorption on the catalyst complex which may operate in the structure of the membrane, this appears more probable than the passage of K^+ ions in association with polar organic acid anions when the membrane is, at the same time, largely impermeable to H^+ and Cl^- ions.

Also the acceptance of H atoms by a redox carrier at a low pH is in better accord with observation than the effective action of dehydrogenases at similar pH levels. At the beginning of the next chapter facts explained by the "redox" theory are summarised. Here, an account is given of four additional and supporting observations which establish also the essential similarity of the acidic process in yeast and gastric oxyntic cells. They may be listed as follows:

1. The effect of azide.
2. The equivalence of H^+ ions removed from within the cell to H^+ ions excreted.
3. The parallel production of extra alcohol produced after azide to the acid excretion inhibited in yeast.
4. The active passage of K^+ ions throughout the whole cell, when K^+ and H^+ ions exchange during fermentation.

1. The Effect of Azide

This remarkable effect, which is shared in lesser degree by dinitro-phenol, by diethyl-dithio-carbamate and sali-

cylaldoxime is such that in small concentrations (N/200) it almost entirely eliminates the acid production by yeast, the production of succinic acid, and the active absorption of potassium. It was introduced by Keilin (1936) as an inhibitor of the cytochrome oxidase system. Its effect on muscle was studied by Stannard (1938) and on yeast by a number of workers (e.g., Winzler, 1944; Winzler, Burg and du Vigneaud, 1944; Spiegelman and Moog, 1945; Spiegelman, Kamen and Sussman, 1948; Clifton, 1946; Brockman and Stier, 1947, 1949; Reiner, 1949). Azide is now a well-known inhibitor of synthesis and growth in yeast. Its inhibitory action on phosphate uptake has been noted by Spiegelman, Kamen and Sussman (1948) as well as its inhibition of the organically bound P increase during fermentation. We have found the effect, on the acid production and K^+ absorption is, however, very much greater quantitatively than that on P uptake.

While azide inhibits the acid-alkali mechanism it has no inhibitory action on the production of alcohol. In fact, as is now well known, the yield of alcohol is increased. It is in this respect it differs so markedly in action from monoiodoacetic acid, which in similar small concentrations entirely inhibits the formation of acid, but at the same time it inhibits the alcohol production with equal effectiveness. The effect of different azide concentrations—without buffering—has been examined, and a concentration as low as 10^{-4} N causes approximately a 50 per cent inhibition.

Azide also inhibits the production of acid by the gastric mucosa after histamine stimulation, while at the same time increasing the oxygen absorption (*Davies, 1949*).

Interpretation of the azide effect on the excretion of acid. This is interpreted as either a direct action on the metal catalyst, which serves as H^+ ion donor or, alternatively, on whatever reactions are required to oxidise

the catalyst within the cell, and which for yeast may require phosphate bond energy.

AZIDE AND THE FORMATION OF ENERGY-RICH PHOSPHATE BONDS

Spiegelman, Kamen and Sussman (1948) have considered the action of the azide to lie in the inhibition of transport of phosphate from 1.3-diphosphoglyceric acid to adenosine diphosphate (ADP), the inhibition occurring by an effect on the combination of 1.3-diphosphoglyceric acid with the transferring phosphoglycerase.

The action is represented as:

$$p\text{-glyceryl} \sim ph \cdot E + N_3^- \rightarrow ph\text{-glyceryl} \sim N_3 + E + ph \quad (5)$$

$$ph\text{-glyceryl} \sim N_3 + OH^- \rightarrow ph\text{-glycerate} + N_3^- \quad (6)$$

In these equations Lipmann's (1941) convention ($\sim ph$) for the energy-rich phosphate bond is used.

The evidence on which this is based is chiefly as follows:

(a) Azide inhibits the transference of inorganic P within the yeast cell to form organically bound P.

(b) Similarities with the effect of arsenate shown by decreasing the sensitivity of the triose dehydrogenase system to iodoacetate and increasing the sensitivity of the fermentation to fluoride.

The effect on the dehydrogenase system in vitro was studied. The reaction system contained crystalline triosephosphate, dehydrogenase, glyceraldehyde phosphate and diphosphopyridine nucleotide (DPN), with sodium pyrophosphate adjusted to pH = 7.4 as the buffer system. The dehydrogenation and acceptance of H atoms by the DPN occurs with added phosphate or with added arsenate which can replace phosphate in the acyl phosphate of

diphosphoglycerate (or phosphate attached to the COOH group).

The results with azide were quite negative. Spiegelman, Kamen and Sussman then concluded that the effect occurred after the attachment of the phosphopherase, and in the manner indicated in the equations above. Both with arsenate and azide the final transference of phosphate to ADP was inhibited, because the arsenate compound of phosphoglycerate broke down rapidly, and azide split the compound of diphosphoglycerate with phosphopherase liberating the acyl phosphate.

Such conclusions for azide are based, however, on negative results in vitro, no direct determinations being made of the effect of azide on the diphospho-glycerate-phosphopherase reaction.

Also azide was found to have no effect in preventing phosphate esterification in cell-free extracts at concentrations capable of complete inhibition of phosphorylation by intact cells.

Reiner (1948) considers that the point of action of azide is other than, or in addition to, its inhibition of the formation of energy-rich phosphate bonds, from a study of the action on adaptive enzyme formation by yeast cells of arsenate and azide. Here adenosine triphosphate (ATP) counteracts the arsenate inhibition but not that of azide.

For the present discussion the effects of azide may be listed as follows:

(a) In general it inhibits synthetic processes of various kinds and this even includes embyronic development in the frog which can proceed anaerobically.

(b) More specifically, it prevents in yeast the change of inorganic to organically bound phosphate within the cell (Spiegelman, Kamen and Sussman, 1948).

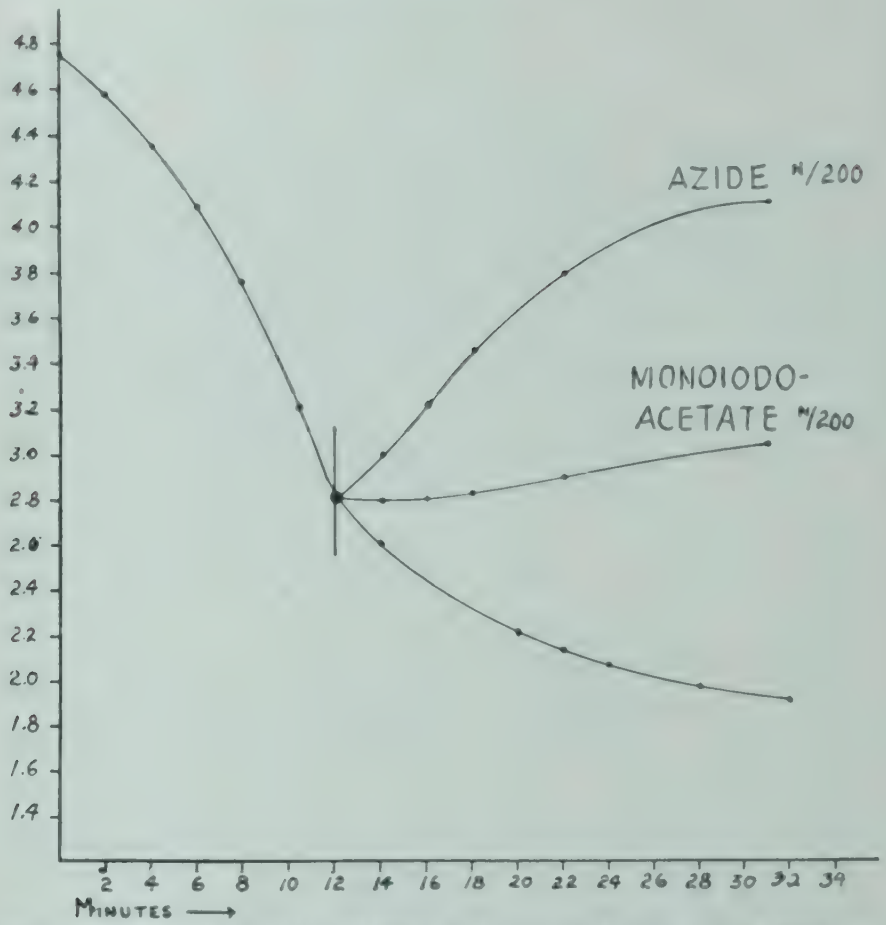

Fig. 17. Effect of azide (N/200) and monoiodoacetate (N/200) on the course of acid production by yeast. Fermenting mixture consisted of one part washed centrifuged yeast to one part of 5 per cent glucose containing M/10 KCl. A very small volume of strong NaN_3 (pH = 2.8) to make N/200 in suspending fluid added when the mixture reached a pH of 2.8. Similarly with monoiodoacetate. The full course of the pH fall was determined in similar mixture without addition of inhibitors (Conway and Carton, 1950).

(c) It inhibits the rapid exchange of K^+ for H^+ ions during short period fermentation (i.e., with high proportion of yeast to suspending fluid). Here 50 per cent inhibition can be produced by as low as 10^{-4} M. azide.

(d) It inhibits the excretion of succinic acid by yeast

and thus along with the previous effect the total acid excretion by yeast is inhibited and without buffering is reduced practically to zero by azide from 10^{-3} to 10^{-4} M. in the suspending fluid.

(e) It completely inhibits the uptake of phosphate in fermentation and even the mixing of external inorganic P with internal (Conway and McDonnell, 1950).

(f) While the acid excretion, including the exchange of K^+ and H^+ ions can be practically eliminated by azide, the yield of alcohol is increased rather than diminished. In a similar way azide entirely inhibits the acid production by the oxyntic cells of the frog, and at the same time the oxygen consumption is increased (Davies, 1949).

(g) *In the resting cell azide does not inhibit the slow entrance of* K^+ *ions* and mixing with the cellular K.

If we consider the effect on the active exchange of K^+ and H^+ ions this might be interpreted indirectly in terms of phosphate bond energy in yeast if the restoration of the catalyst to the oxidised condition is dependent thereon. In Figure 17 is shown the effect on the pH curve of adding azide (to form M/200 strength) to a fermenting mixture (1 kg. yeast to 1 litre of 5 per cent glucose containing M/10 KCl) some time after the onset of the fermentation. The pH of the sodium azide added was the same as the pH of the mixture at the time of addition.

It will be seen that not only is the effect practically instantaneous, but that H^+ ions return rapidly to the cell. Analysis of the mixture shows that this occurs as an exchange of K^+ for H^+ ions.

There is a rather striking contrast between the azide effect and that of monoiodoacetate as shown in Figure 17.

If no KCl was present in the suspending fluid, and azide added as before, the succinic acid excreted into the suspending fluid is changed to potassium succinate, also by

exchange of K^+ and H^+ ions, but there is no fall or change in the concentration of the succinic system. Neither succinic acid nor succinate return to the cell.

The azide effect added after fermentation may constitute a true reversal, with displacement of the dynamic balance of the catalysts involved. This, which would be of great interest, is being investigated at present.

EFFECT OF OTHER INHIBITORS

2.4-dinitro-phenol like azide (but in lesser degree) inhibits the acid production but does not inhibit the alcohol formation. Similarly salicyl-aldoxime and diethyl-dithiocarbamate inhibit somewhat the production of acid. The effect of the diethyl carbamate which is an efficient inhibitor for copper suggests that the metal involved may not be Fe.

2. The Equivalence of H^+ Ions Removed from Within to H^+ Ions Excreted both for the Yeast and the Gastric Oxyntic Cell

Concurrent with gastric secretion, total base and CO_2 combining power were shown to increase in the blood by Bulger and Allen (1927); Bulger, Allen and Harrison (1928); and by Brunton (1933). Similar results were obtained by Hanke, Johannesen and Hanke (1931) who were able to demonstrate that the amount of acid liberated by the gastric tissue during secretory activity was approximately equivalent to the amount of alkali formed simultaneously. As quoted by Hollander (1943) this observation had been anticipated by Brücke as far back as 1859. For the isolated frog stomach the experiments of Davies (1948) indicate a similar equivalence.

If the underlying mechanism of acid secretion is the same for the yeast cell as for the parietal cell, the equiva-

lence of alkalinisation to acid secreted should likewise occur. To demonstrate this, however, it is essential to examine short period fermentations in which a high proportion of yeast cells to fluid (such as 1:0.6 or 1:1) is used, and the amount of new succinic acid formation is relatively small. Where a small amount of yeast is used to a large amount of fluid, as is customary, a relatively considerable amount of succinic acid is formed *de novo* from glucose as compared with the amount in the cells and as first shown by Kleinzeller (1941).

In the investigation with yeast two methods were used. In the first the interspace water was pressed out after centrifuging and then the yeast cells smashed by freezing with liquid air and subsequent thawing. The acid required to titrate back to the pH of resting yeast (5.8) was determined.

In the second method the whole suspension was frozen in liquid air, then thawed, evacuated of gas, and the amount of alkali required to bring to the pH of a control with resting yeast, similarly treated, was observed. Usually little was needed, and from a knowledge of the external acidity, the alkali formed within the cells could be calculated. The result of such investigations showed that as an average about 93 per cent of the excreted acid is associated with an equivalent alkalinisation within the cells.

The equivalence of alkali to acid produced holds whether the acid is excreted as succinic acid or whether the H^+ ions are exchanged for K^+ ions.

This last fact along with the azide effect of inhibiting with equal effectiveness the secretion of succinic acid or the H^+ ions resulting from an exchange of K^+ and H^+ ions, show that both these forms of acidity derive from the same source. In other words, the H^+ ions that unite with succinate ions or exchange for K^+ ions have the same origin.

3. The Parallelism between the Extra-Alcohol Produced and the Acid Inhibited in the Presence of Azide

For the conditions we have studied, centrifuged yeast and suspending fluid containing 5 per cent glucose, being in a 1:3.4 relation, there appears to be a certain parallelism between the acid inhibited by azide and the alcohol formed, one being decreased and the other increased. Initially (Conway, Brady and Carton, 1950) this seemed to go in a relation of an increase of 1 mol. alcohol to 2 mols. of acid inhibited, but a fuller investigation showed that the conditions could be varied to obtain a change in the alcohol formation much greater than this relation would imply.

The conditions were either definitely anaerobic, with cyanide, or largely anaerobic with rapid fermentation in a container with relatively small outlet and no oxygenation.

For the parietal cells 10^{-4}M. azide inhibits the acidic formation and increases the oxygen consumption (Davies, 1949). Here the experiments of Clifton (1946) with regard to the effect of azide on yeast fermenting aerobically, indicate that with 10^{-4}M. azide a slight increase of oxygen consumption can occur. With the yeast used and without inhibitor only a small fraction of the glucose was converted to alcohol. With the azide, this fraction was much increased.

In general, and with a relatively small proportion of yeast to suspending fluid, there appears to be a more rapid anaerobic fermentation in the presence of azide, which Spiegelman *et al.* (1948) have interpreted as due to the uncoupling of a dehydrogenation process with phosphate transference, and the release from the inhibiting effect of limited phosphate acceptors. With high propor-

tion of yeast to suspending fluid, we have not found that azide increases the rate of alcohol production, but it does increase the final amount (Fig. 18).

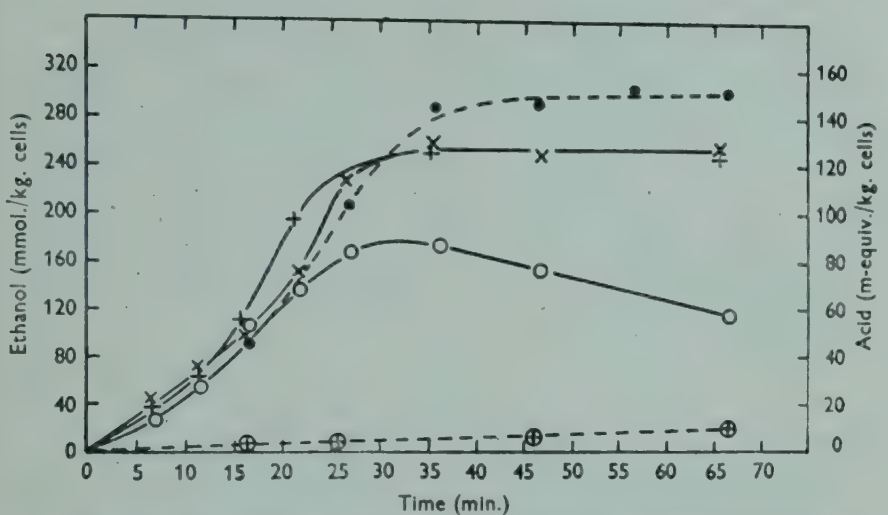

Fig. 18. Mean curves of acid (O) and of alcohol production with and without N/200 cyanide (+, ×). No difference in the acid production was observed due to the cyanide (Fermenting mixture: 1 kg. washed centrifuged yeast to 3.4 litres of 5 per cent glucose). The acid and alcohol production with azide (⊗, ●) are also shown. (Conway, Brady and Carton, 1950; Courtesy of *Biochem Journal*.)

4. The Active Passage of K^+ Ions into the Whole Yeast Cell

With an exchange of K^+ and H^+ ions during fermentation of about 30 m.eqs./kg., if all the K^+ ions were retained in the outer region of the cell, it would reach a concentration of about 300 m.eqs./kg. Also the associated anions would have a similar concentration. In fact, however, the K^+ ions pass rapidly into the whole cell. This is shown by using the azide effect some time after the onset of fermentation. As already mentioned, the same quantity of K^+ ions absorbed during the fermentation now come out rapidly in exchange for H^+ ions. If the entering K^+

ions are labelled with K^{42} and remain in the outer region, the returning K^+ ions should give approximately the same specific count as the K^+ ions absorbed. The count, however, is greatly decreased and to an extent which shows mixing with the whole quantity of K^+ ions of the cell.

It would appear then that with H^+ exchanging for K^+ ions, in the acidic region, the latter are brought rapidly across the membrane along with the reduced form of the heavy metal catalyst, however the passage of this latter be envisaged. Such passage may be only a rotation due to thermal agitation of a kind considered by Lundegårdh for plant root hairs.

SUMMARY

The organic acid theory in the form of a reduced heavy metal catalyst acting as H^+ ion donor, and operating in a redox cycle across a membrane interprets the facts more satisfactorily than the organic acid as metabolite. An outline of such a redox theory is given.

Two additional facts illustrate similarity of the production of acid in yeast and in the parietal cell. Thus, azide (and also 2.4-dinitrophenol) can completely inhibit the acid production in yeast and at the same time increase the amount of alcohol formed.

These same inhibitors can, in a similar manner and in suitable concentration, inhibit the acid production while increasing the oxygen consumption in the isolated gastric mucosa of the frog (Davies, 1949).

Also, for each equivalent of H^+ ions secreted by the active parietal cell or by the fermenting yeast cell (concentrated suspension) there occurs an equivalent intracellular alkalinisation or removal of H^+ ions.

CHAPTER VI

DEVELOPMENT OF THE "REDOX" THEORY: OTHER THEORIES OF HCl SECRETION

WHAT THE "redox" theory explains may now be summarised.

With a metal catalyst as donor—it explains:

(a) How H^+ ions can be excreted in high concentration without their passing directly from the cellular fluid across a very high concentration difference.

(b) Why no indication of a metabolite of glucose acting as a direct H^+ donor can be found for the production of parietal or yeast acidity.

(c) How H^+ ions can be formed metabolically at a low pH.

(d) Why for each equivalent of H^+ ions excreted a H^+ ion equivalent is removed from within the cell.

(e) The action of azide in inhibiting the acid secretion from the parietal or oxyntic cell, while increasing the oxygen consumption; and inhibiting the yeast acidity while increasing the alcohol production.

(f) The action of other inhibitors, such as monoiodoacetic acid which, cutting off the entire supply of H atoms from dehydrogenase, eliminates the formation both of acid and alcohol in yeast. It also explains why cyanide (N/200) completely inhibits H^+ ion secretion in the parietal or oxyntic cell, by blocking the path of the electrons to

oxygen, but has no appreciable effect on H^+ ion excretion by anaerobically fermenting yeast.

(g) Why HCl, once formed, cannot readily return.

(h) How the secretion of HCl from the parietal cell can be quantitatively greater than the oxygen consumed.

(i) To the above it may also be added that it explains the striking similarity of acidic production by the parietal cell and the yeast cell when fermenting under special conditions.

With a metal catalyst as carrier—it explains why

(j) K^+ ions are actively brought across the membrane of the yeast cell, by displacement of H^+ ions on the surface of the catalyst, and the formation of an adsorption complex.

(k) Possibly the active secretion of succinate ions may be interpreted in similar fashion.

THE PARALLEL BETWEEN YEAST CELL AND PARIETAL CELL AND THE ACTIVE ABSORPTION OF POTASSIUM IONS

The fact that the highest concentrations of free H^+ ions appear during fermentation as a result of an exchange of K^+ and H^+ ions may (as already noted) obscure a more significant analogy between the excretion of succinate ions and of Cl^- ions.

For the yeast cell the H^+ ions come from the same source whether succinic acid is excreted—as in the absence of K^+ ions—or is almost all absent after prolonged oxygenation, and the lowest pH values (as low as 1.4) obtained by an exchange of K^+ and H^+ ions.

This is shown by the effect of azide, which can inhibit almost completely the excretion of succinic acid or the H^+ ions exchanging with K^+ ions; also from the fact that whether the acidity outside the cell is entirely due to suc-

cinic acid or to free H^+ ions, there is an equivalent alkalinisation within the cell using a concentrated suspension.

If then in yeast without added KCl only succinate ions are available in quantity to accompany the H^+ ions liberated by the ionization of the reduced metal catalyst, while in the parietal cell Cl^- ions play the same role, the significant parallel may lie between the secretion of succinic acid by yeast and HCl by the parietal cell.

FURTHER DEVELOPMENT OF THE "REDOX" THEORY

It will appear from the foregoing account that the general principle of a metal catalyst operating in a redox cycle across a membrane—here the inner membrane of the acidic region—covers the facts better than an organic acid as a metabolite operating also in a cycle, and in fact so well that some confidence may be entertained that it constitutes the essential acting mechanism.

The more detailed formulation involves working hypotheses liable to change on further investigation. Here some considerations are advanced concerning the nature of the catalysts involved, followed by a tentative formulation of the stages of the cycle.

Steps in the Transfer of H Atoms to the Metal Catalyst

These, for the present purpose, may be taken to involve the initial organic substance or substances yielding the H atoms, pyridine nucleotides, flavine enzymes and the metal catalysts.

It is unlikely that H atoms pass directly from the metabolite molecules to the metal atoms (though in the case of lactic dehydrogenase from yeast there is evidence to show that the enzyme contains cytochrome-b_2 as part of

molecule according to Bach, Dixon and Zerfas, 1946, and no pyridine nucleotide or flavine mediator is required).

When two H atoms are received by cozymase, or other pyridine nucleotide, one of them (at cellular pH levels) is changed to an H^+ ion, the electron being retained (Karrer, *et al.*, 1936; Warburg and Christian, 1936).

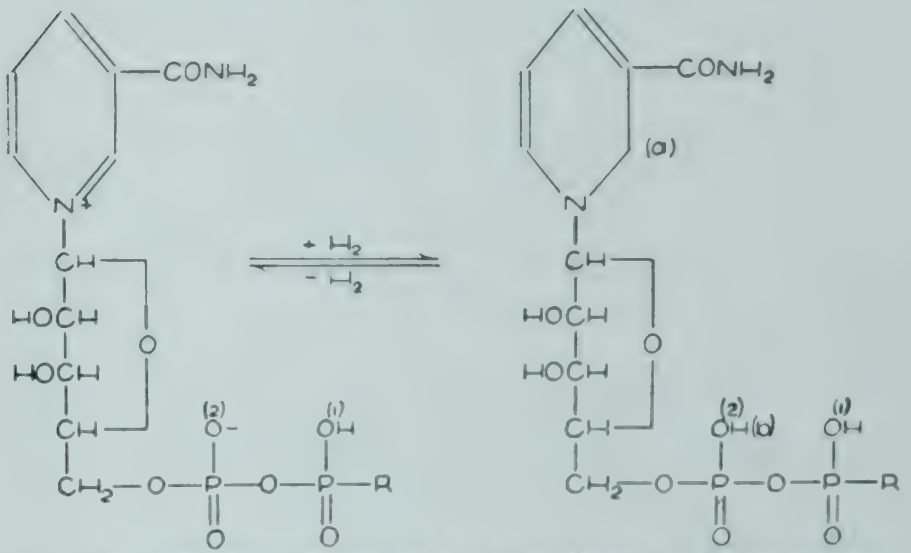

Fig. 19. The formulae indicate the interchange of H atoms with cozymase, in a redox change. At intracellular pH values the OH groups attached to P are ionized in both reduced and oxidised forms. In the oxidised form the negative charge at position "2" is balanced by the positive charge on the N atom of the pyridine ring, and when this disappears in a reduced form an extra negative charge appears on the molecule and a H^+ ion is released into the solution.

In Figure 19 is shown the change involved in the acceptance of hydrogen by diphosphopyridine nucleotide. The N atom of the pyridine nucleus changes its valency and a H atom is accepted in the ring in position a. The other is accepted in position b. The acidic group at 1 has a pK value of approximately 4.7 and at 2 the pK appears to be less than 3.0 (according to Meyerhof as quoted by Schlenk, 1949).

Thus, both these groups will be completely ionized at cellular pH values, and one of the H atoms accepted by the molecule will have contributed a H^+ ion to the solution.

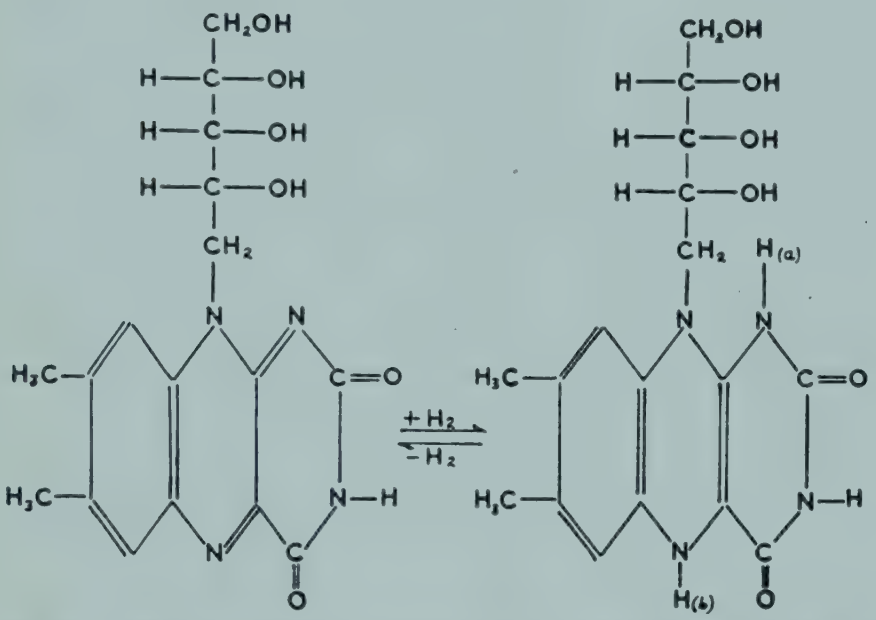

Fig. 20. This indicates the positions of the H atoms released or taken up in the redox cycle of riboflavin.

At the next stage of acceptation of H atoms by flavine enzymes, a H^+ ion will be taken up again along with the electron passing from cozymase.

The transfer of H atoms is shown in Figure 20 for riboflavin (F).

One may write:

$$C\bar{o}H + F + H^+ = Co + FH_2 \qquad (1)$$

For the acceptation of H atoms by the metal catalyst, one may write in turn:

$$FH_2 + 2M^{(n)+} = F + 2M^{(n-1)+} + 2H^+ \qquad (2)$$

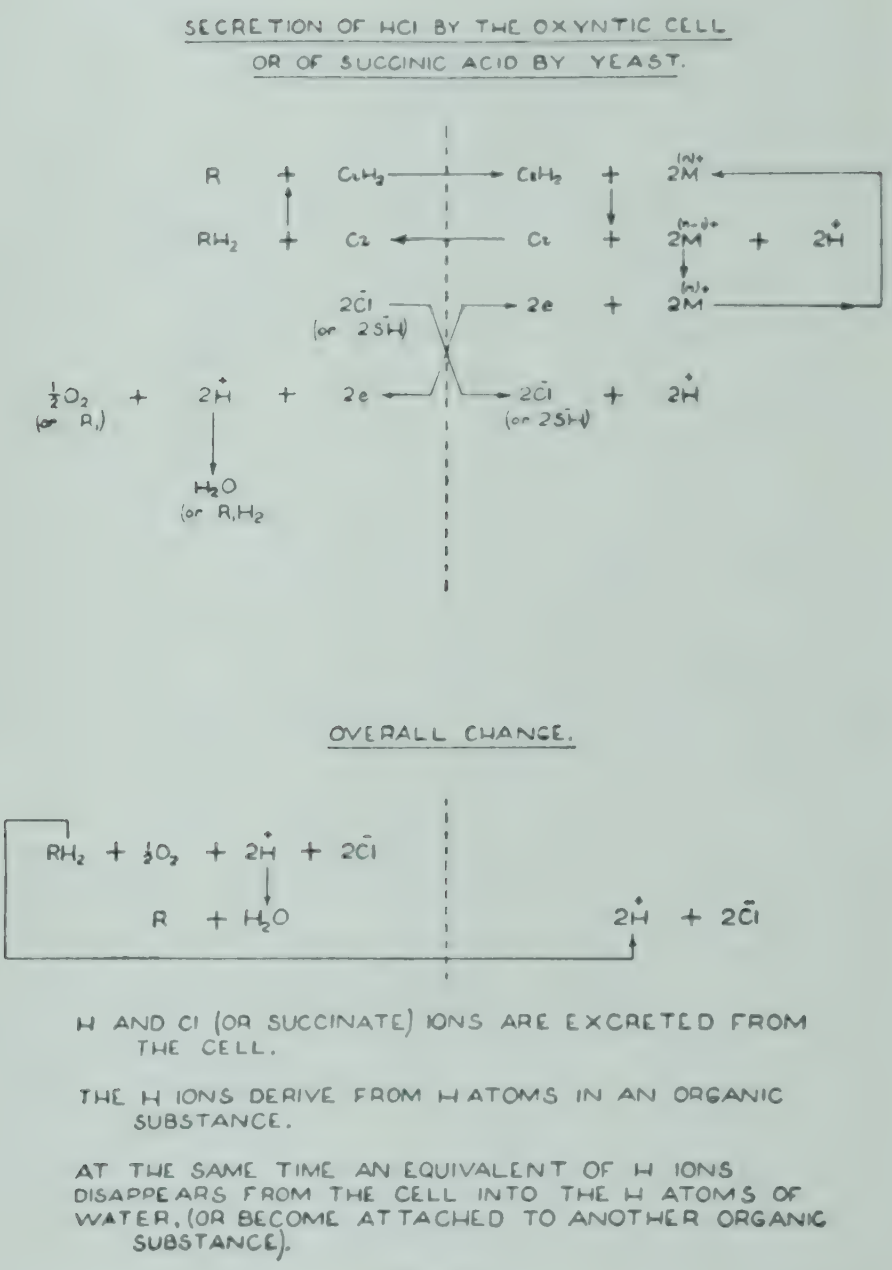
SECRETION OF HCl BY THE OXYNTIC CELL
OR OF SUCCINIC ACID BY YEAST.
R
RH2
C1H2
C2
2Cl⁻
(or 2SH)
2e
2M
2H⁺
½O2
(or R1)
H2O
(or R1H2)
OVERALL CHANGE.
RH2 + ½O2 + 2H⁺ + 2Cl⁻
R + H2O
2H⁺ + 2Cl⁻
H AND Cl (OR SUCCINATE) IONS ARE EXCRETED FROM THE CELL.
THE H IONS DERIVE FROM H ATOMS IN AN ORGANIC SUBSTANCE.
AT THE SAME TIME AN EQUIVALENT OF H IONS DISAPPEARS FROM THE CELL INTO THE H ATOMS OF WATER, (OR BECOME ATTACHED TO ANOTHER ORGANIC SUBSTANCE).

Fig. 21.

In the equation 'n' represents the total charge on the whole metal catalyst molecule or complex.

Dealing only with the above catalysts, it would seem

necessary for the full efficiency of conversion of H atoms to H^+ ions, that either the dehydrogenase, flavoprotein and metal catalyst operate in the acidic region, with subsequent oxidation of the reduced metal catalyst in the central region; or, alternatively, that the stages up to flavoprotein are carried out in the central region.

Tentative Formulation of Stages in the Cycle

The stages of the secretion of HCl by the oxyntic cell or of succinic acid by yeast are presented in Figure 21. The processes are very similar except that in the animal cell oxygen is the final acceptor of the electrons and in yeast an organic substance. For the fate of the metabolic H atoms involved one may write simply:

$$RH_2 \rightarrow 2H^+ + 2\epsilon + R \qquad (3)$$

$$2\epsilon + {}^1/_2O_2 + 2H^+ \rightarrow H_2O \qquad (4)$$

or,

$$2\epsilon + R_1 + 2H^+ \rightarrow R_1H_2 \qquad (5)$$

Under Figure 21 is also given the overall summary.

The following stages may then be distinguished.

First Stage. A metabolite, RH_2, is acted on by a dehydrogenase, and H atoms transferred to cozymase. In this process an energy-rich phosphate bond may be considered transferred to ADP, or other acceptor.

This stage is considered to take place essentially in the inner region of the cell, and may be written simply:

$$RH_2 + Co \rightarrow R + C\bar{o}H + H^+ \qquad (6)$$

Second Stage. The $C\bar{o}H$ transfers its H atom with an electron plus H^+ ion to the metal catalyst either directly or via another stage such as that of a flavine.
The reaction may be written:

$$H^+ + C\bar{o}H + F \rightarrow FH_2 + Co \qquad (7)$$

This stage is considered to occur in the central region.

The flavine molecule now transfers H atoms to the metal catalyst, perhaps via flavoprotein in cell wall:

$$FPH_2 + 2M^{(n)+} \rightarrow FP + 2M^{(n-1)+} + 2H^+ \qquad (8)$$

or, the total transfer up to this stage may be written as in Figure 21:

$$CtH_2 + 2M^{(n)+} \rightarrow Ct + 2M^{(n-1)+} + 2H^+ \qquad (9)$$

which has likewise the merit of not specifically designating the H atom carrier.

Third Stage. Here the process can be regarded from two possible aspects. In the first electrons are transferred across the membrane, with compensatory anion flow in the opposite direction.

In the second, there is a diffusion of the reduced metal catalyst across the membrane, or, at least, a physical displacement as by thermal agitation or the like, in the membrane, so that it acts centrally.

The first possibility is represented in Figure 21. The second is included in Figure 22 representing the active transport of K^+ ions in fermenting yeast when KCl is included in the suspending fluid. In this case the total charge on the electro-adsorption complex is considered to be zero.

Fourth Stage. The passage of the anion outwards across the membrane. This may be considered as intrinsically linked with the third stage and as represented in Figure 21. The actual pull on the anion would then be of the nature of a potential gradient, and the transfer need occur only unidirectionally.

Alternatively an adsorption complex, with the oxidized catalyst more or less specific, and having an overall neutrality could be represented as actively transferring the anion across the membrane. The complex is dissociated

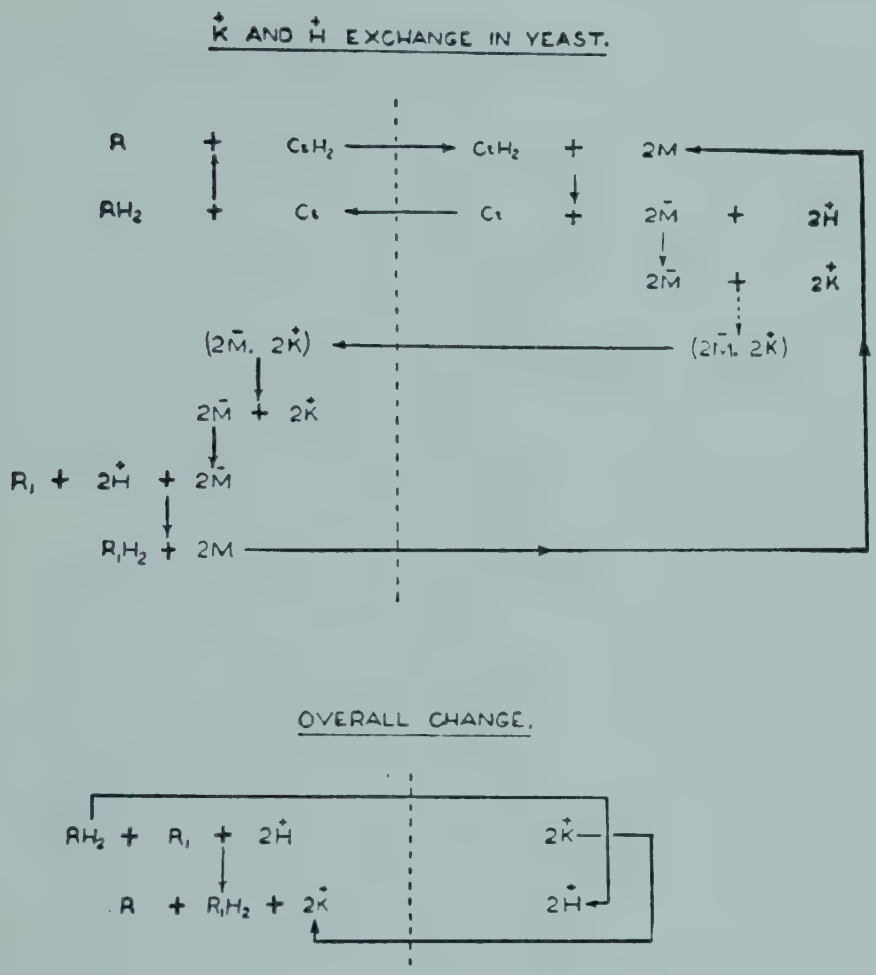
K AND H EXCHANGE IN YEAST.
R
RH2
C6H2
C6
2M
2H
2K
(2M. 2K)
R1 + 2H + 2M
R1H2 + 2M
OVERALL CHANGE.
RH2 + R1 + 2H
R + R1H2 + 2K
K IONS HAVE DISAPPEARED OUTSIDE THE CELL AND BEEN REPLACED BY H IONS.
THE H IONS HAVE DERIVED FROM H ATOMS IN AN ORGANIC SUBSTANCE.
AT THE SAME TIME H IONS ARE REMOVED FROM THE CELL AND APPEAR AS H ATOMS ON ANOTHER ORGANIC SUBSTANCE.

Fig. 22.

in the acidic region as the catalyst becomes again reduced. (Such a process is indicated in Fig. 22.)

Fifth Stage. The transfer of electrons and H^+ ions within

the cell. These are transferred finally to oxygen in the parietal cell but to another organic acceptor in yeast fermenting anaerobically. *The removal of H^+ ions from the cell thus occurs in an equivalent relation to the H^+ ions secreted.*

Sixth Stage. In the yeast cell, the organic substance R_1H_2 could conceivably be changed by one or more steps involving energy input, or the use of phosphate bond energy into RH_2 once more and feeding the acidic cycle in turn. In this way though the immediate transfer across the membrane *would never be H^+ ions but metabolic H atoms, the latter would derive in part from H^+ ions within the cell.*

In the parietal cell a similar process could occur in which an organic acceptor received part of the electrons proceeding from the special metal catalyst along with H^+ ions from the cell to form RH_2 (with energy expenditure) and contribute metabolic H atoms to the acidic cycle.

It has been advanced that *more* than four times the equivalent of H^+ ions are formed by the parietal cell for each mole of oxygen consumed (Crane and Davies, 1948). This conclusion is open to criticism as considered in a later chapter; but if the ratio is in fact greater than 4, then such a cyclical utilisation of an H atom acceptor as in stage 6 may come into action.

A similar concept has been put forward by Davies and Ogston (1950), as their Mechanism 2. Their Mechanism 1 corresponds largely in principle to the scheme as presented above, with cytochrome c as the probable metal catalyst (M). Their mechanism 2 may be given as follows. H^+ ions are taken up from the cell and transferred as H atoms to an organic acceptor:

$$2Fe^{++} + 2H^+ + R \rightarrow 2Fe^{+++} + RH_2 \qquad (10)$$

Since the redox potential of the acceptor system would

probably be much less than the cytochrome system, very little RH_2 would form, but if it is phosphorylated as by creatine phosphate (CrPh) then:

$$RH_2 + CrPh \rightarrow RH_2Ph + Cr \qquad (11)$$

would occur with accumulation of RH_2Ph, provided the phosphate bond of RH_2Ph was less rich in energy than that of CrPh.

The RH_2Ph compound is now considered to diffuse into the acidic region (here so termed) and splits into RH_2 and Ph. The RH_2 is then acted upon by Fe^{+++}:

$$2Fe^{+++} + RH_2 \rightarrow 2Fe^{++} + 2H^+ + R \qquad (12)$$

R and Ph diffuse back across the membrane and the cycle is complete.

OTHER THEORIES OF THE MECHANISM OF HCl PRODUCTION IN THE GASTRIC MUCOSA

Only a brief reference can be made, and views already withdrawn directly, or by implication, or which are now obviously untenable are not considered.

Among these discarded views may be placed the possible use of the urea *forming* mechanism as a direct source of H^+ ions. The true significance of the comparatively large urea metabolism of the gastric mucosa (FitzGerald, *et al.*, 1946–1950; Glick, 1950; Hastings, *et al.*, 1950) is dealt with in Chapter XI.

Secretion of a Neutral Precursor by the Parietal Cell

Bensley and co-workers (Harvey and Bensley, 1912; Hoerr, 1936; Zimmermann, 1925; and Ostrouch, 1937) suppose that a precursor such as a protein hydrochloride is secreted by the parietal cells, with subsequent hydrolysis.

Such experiments are largely based on observations of

the pH in the canaliculi or cells with indicators, and after the killing of the animal. The rapidity of diffusion over the small distances concerned, the fact that the cell itself becomes more alkaline on secreting acid, as may be readily shown for the yeast cell; also, the probable alteration in permeability on the cessation of the oxygen supply to the mammalian tissues, make such observations of very little value. To this one may add that the process of fixation, even with the Altmann-Gersh freezing-drying technique, may be expected to produce great changes in local concentrations of HCl.

Hollander has pointed out "that the *higher* the concentration of HCl in pure gastric secretion from dogs, the *lower* the concentration of all other substances" and this alone is sufficient to outrule the primary secretion being a neutral complex chloride.

Hollander's Views on the Manner of HCl Formation

Hollander considers the cell wall of the canaliculi as permeable only to H^+ ions and Cl^- ions, and that the free acid first occurs either in the cell wall or in the cytoplasm immediately adjacent to the intracellular canaliculus. Hollander's views (1943 and 1949 reviews) with respect to the intimate nature of HCl secretion may briefly be described as follows.

He considers that the wall of the intracellular canaliculus is a membrane which is "*irreciprocally permeable* only to water (and therefore H^+ ion) and to the Cl^- ion (and the other halides)."

When a secretory stimulus is applied, fluid passing forward into the intracellular canaliculus will carry H^+ ions and Cl^- ions with it "thus effecting a *membrane hydrolysis* of the neutral chlorides with simultaneous separation of the HCl so formed." Isotonicity of the HCl secreted with the

blood or parietal cell, he considers may well be related to the minimizing thereby of the total work done in secretion.

The author's views are in agreement with those of Hollander with respect to the emphasis on the wall of the canaliculus (and possibly of the cell wall itself in the Batrachial gastric glands), and the isotonicity of the parietal secretion. There is some difference concerning the K^+ content, basing this on the work of Gray (1943) and the analysis of human data by Fisher and Hunt (1950). At the same time it may be said that such phrases as "irreciprocally impermeable" or "membrane hydrolysis" in themselves do not carry the solution of the intimate mechanism any real step forward, though no doubt intended as merely empirically descriptive statements.

Other "Redox" Views: Theory of Davies *et al.*

Shortly after the publication of the author's "redox" hypothesis (Conway and Brady, 1948), Crane and Davies (1948) and later Davies and Ogston (1950) published a similar interpretation of the essential reaction in H^+ ion secretion. The present writer's views would seem to differ in the following respects.

(a) The recognition of a distinct acidic region, with membrane barrier preventing the return of free HCl.

(b) The view that the H^+ ions derived from the oxidation of glucose or its metabolites would appear to suffice without recourse to a second cyclical mechanism. The total carbohydrate metabolism may be available to the oxyntic cell, and its metabolism exceed greatly that of the other cells in the glandular tubule. If necessary, however, such a cyclical mechanism can be considered as subsidiary to the main process and operate as described above.

(c) The view that the secretion of a slightly hypertonic HCl solution would suffice for the secretion of water by

osmosis, and that the isosmotic view applies even to Teorell's glycine experiments. The secretion of water as a necessary result of the secretion of HCl was first proposed by the present writer (Conway, 1949), though the osmotic equivalence of the parietal secretion with the blood was a view long entertained. From the data of Teorell and co-workers this equivalence did not appear to be a necessary relation, but it has been shown in Chapter II that their figures only support the isosmotic view when more fully considered.

(d) A somewhat different treatment of the energy question as considered later.

The course of Davies' views are of some interest here, in connection with the organic acid hypothesis, of which the "redox theory" is a variant. As already indicated he obtained a Q_{HCl}/Q_{O_2} quotient greater than unity for the isolated gastric mucosa of the frog, and after histamine stimulation. He considered this to disprove the organic acid theory. It was pointed out that this conclusion was incorrect (Conway and Brady, 1947) as the acid might be cyclically reformed, with the available energy only as the limit to the quotient.

The reply to this was, in effect, that in the reconstitution of the organic acid, water would have been incorporated, and that the H atoms in the water so taken up would supply the H^+ ions of the gastric juice. Davies would have it that the H^+ ions of the gastric juice were provided by the "splitting" of water. While this uptake of water would apply to acids of the cyclically reformed metabolite type, it is obvious, nonetheless, that he was confusing the significance of immediate and remote origins. The significant process here is the high local concentrating of H^+ ions, and not their remote origin. In fact, all the H atoms in all the carbon compounds in nature derived pri-

marily from the photosynthetic process, come from water, but such an origin sheds no light on such problems as the concentrating of H^+ ions by the oxyntic cells.

Having arrived at the conclusion that a cyclically reformed organic acid was, from our standpoint, the best working hypothesis, it appeared that under this heading could be included the transfer of H atoms to a metal catalyst which at once ionizes, producing H^+ ions and retaining the electrons. This would give a Q_{HCl}/Q_{O_2} quotient of 4.0, and could account fully for the respiratory data with the frog's mucosa (Davies, 1948) if all the oxygen consumed by the oxyntic cells be taken into account. This is less than the total oxygen uptake, but it may be very little less for the frog's mucosa in which the acid-forming cells are in high proportion and with the metabolism of the oxyntic cells probably greater than the other cells of the mucosa. In this way *all* the H^+ ions secreted could have come from H atoms in metabolites of glucose, and 50 per cent from H atoms in the original glucose. Thus the repeated statement of Davies *et al.* that "the hydrogen ions of the acid could not be produced directly by the fermentation or oxidative degradation of any carbon compound" is shown to be incorrect.

Lundegårdh's scheme (1946) for active anion absorption in root hairs (especially with the modification proposed by Robertson and Wilkins 1948a, 1947b) has some resemblance to the "redox theory" for H^+ ion secretion but is essentially very different, and it would seem appropriate to consider its origin and present significance.

Following the theoretical development begun by Lund and Kenyon (1927, 1928) and continued by Stiehler and Flexner (1938) for redox cycles in cell surfaces, Lundegårdh (1946), as already indicated, advanced a view as to the mechanism of active anion absorption in root hairs.

This was related to the following reaction:

$$\begin{pmatrix} Fe^{+++} \\ 3A^- \end{pmatrix} \overset{+\,\epsilon}{\underset{-\,\epsilon}{\rightleftarrows}} \begin{pmatrix} Fe^{++} \\ 2A^- \end{pmatrix} + A^-$$

the iron being that of the cytochrome-cytochrome oxidase system. In this way anions are transported in the direction of lesser redox potential. They were represented as moving across "adsorption tracks" or ionised chain molecules. In this way potentials are produced which attract cations. "The absorbed metallic cations are liberated (exchanged for H^+ ions) at the points or surfaces of anion accumulation. New cations are absorbed from the medium and transported owing to their concentration and mobility. The anion respiration thus acts as the promoter of salt accumulation even if the cations are more passively dragged through the protoplasm." It will appear from this, that as a stream of metallic cations are absorbed passively, and M^+ and A^- ions enter the cell in equivalent relation, there is no place for any surplus liberation of H^+ ions. The point concerning the exchange of metallic ions for H^+ ions is either unnecessary, or may be considered at the beginning as a minute fraction of the whole cation transport, the cations following successively into place in the direction of transport, and doing so subject to the potential pull.

Robertson and Wilkins have modified this scheme (1948a).

They have taken the unnecessary H^+ exchange part and elaborated it into what is virtually active cation as well as active anion absorption. The cations are now absorbed by a reduced metallic catalyst, this being formed in accordance with the reaction:

$$RH_2 \rightarrow R + 2H^+ + 2\epsilon$$

The cations outside exchange for these H^+ ions and H^+ ions appear outside the cell in the Robertson and Wilkins scheme (1948a). At the same time Cl^- ions are actively absorbed in accordance with Lundegårdh's scheme. Thus K^+ and Cl^- ions enter in equivalent relation and H^+ ions appear outside. It should be obvious that H^+ ions could not accumulate here because of unbalanced charges.

But it may be added that they were not directly concerned with H^+ ion secretion, still less with secretion from the oxyntic cells of the gastric mucosa, but rather with a quantitative investigation of the Lundegårdh hypothesis of salt transport in root hairs.

In short, neither in the Lundegårdh scheme nor in the modification advanced by Robertson and Wilkins could H^+ ion accumulation appear outside the cell. Also, it has long been known to biochemists that such reactions as

$$RH_2 + 2Fe^{+++} \rightarrow R + 2Fe^{++} + 2H^+ + 2\epsilon$$

and

$$2H^+ + 2\epsilon + \tfrac{1}{2}O_2 \rightarrow H_2O$$

occur in the cell (though the second reaction may be differently expressed). The net result with respect to H^+ ion formation is that in general though there are considerable H^+ ion exchanges the cell remains neutral. The essential nature of the "redox theory," as here proposed, is that the first of these processes occurs where the H^+ ions are prevented from returning to the cell by a membrane barrier, and anions are brought out either by the p.d. developed or in turn actively extruded.

Such a theory was outlined from the author's laboratory and it does not seem possible that any high local concentrating of H^+ ions could occur, with the efficiency experimentally found without a definite phase boundary or membrane separating them from the general protoplasm.

Rehm's Theory (1950). Rehm is in agreement with

the author's view as to the origin of the H^+ ions, but includes gastric electromotive forces as acid-controlling mechanisms for the secretion. This is discussed later.

Theory of Patterson and Stetten (1949). These workers consider that cozymase plays the role of H^+ ion donor in the reduced state, rather than a metal catalyst. Objections to such a view are as follows:

(a) As discussed in a later chapter, the energy released up to the pyridine nucleotide stage would be insufficient to account for the high chemical potential of the H^+ ions, if these are secreted with Cl^- ions, in almost isosmotic concentration with the blood, and when only 50 per cent of the metabolic H atoms are, at most, available.

(b) Azide in suitable concentration (Davies, 1949) can eliminate the secretion of HCl and increase the consumption of oxygen, just as in yeast it can prevent the secretion of H^+ ions and increase the alcohol output, but azide in the concentrations used has no effect on the "redox" change of cozymase.

SUMMARY

A summary of facts connected with the secretion of acid is given and these are explained by the "redox" theory.

A formulation of the scheme for HCl secretion by the oxyntic cell and of succinic acid by yeast is given; also for the exchange of K^+ and H^+ ions in fermenting yeast and the active transport of K^+ ions.

A brief consideration of other theories of HCl secretion is given, and of "redox" theories by Crane and Davies (1948), by Davies and Ogston (1950), by Rehm (1950) and by Patterson and Stetten (1949).

CHAPTER VII

ENERGETICS

THE REVERSIBLE work ΔW done in obtaining a litre of fluid containing a solute A, in concentration C_{A_1} from a very large amount of fluid containing the solute in concentration C_{A_0} may be expressed as:

$$\Delta W = RT[C_{A_1} \, ln \, (C_{A_1}/C_{A_0}) - C_{A_1} + \Sigma C_0] \qquad (1)$$

The expression ΣC_0 represents the sum of all the solute concentrations in the large amount of fluid.

More generally, if the litre of fluid contains A, B, C, etc., solute molecules, the reversible work done is given by:

$$\Delta W = RT[\Sigma C_{A_1} \, ln \, (C_{A_1}/C_{A_0}) - \Sigma C_{A_1} + \Sigma C_0] \qquad (2)$$

In the special case where the secreted fluid is isosmotic with the mother fluid, then the two members to the right in equations 1 and 2 cancel out.

If the solutions contain ions instead of neutral molecules the form of the equation remains the same, since no electrical work is done, whatever potential may exist across the membrane (the electrical work performed for the transfer of cations being equal to and opposite that for the anions). In equation 2 it is only necessary to regard each ion species as if it were a molecular species, with activities substituted for concentrations.

Hill's solution of the problem and with respect to urinary secretion (as given in Barcroft's *Respiratory Function*

of the Blood, 1914, and in *Principles of General Physiology* by Bayliss, 4th Edition, 1924) is somewhat different. His equations with the same symbols as above may be written:

$$\Delta W = RT[C_{A_1} ln\ (C_{A_1}/C_{A_0}) - C_{A_1} + C_{A_0}] \quad (3)$$

$$\Delta W = RT[\Sigma C_{A_1} ln\ (C_{A_1}/C_{A_0}) - \Sigma C_{A_1} + \Sigma C_{A_0}] \quad (4)$$

The difference is in the last member on the right of the equation. Hill's equation 3 represents, like equation 1, the reversible work done in separating one litre of fluid containing a solute in concentration C_{A_1} from a large volume of mother fluid containing the solute in concentration C_{A_0} but with the added condition that *all other solutes in the litre of separated fluid are in the same concentration as in the mother fluid.* A similar limitation applies to his more general equation 4. In this all other solutes of the mother fluid apart from those considered to be actively secreted must be present in equal concentrations across the membrane.

If, as in the case of the parietal secretion, Na^+ ions as well as other plasma constituents are absent, equation 4 is inapplicable, also if such a secretion were isosmotic with the blood, the two members to the right of equation 4 would not cancel out, and very erroneous calculations could result. The following gives a derivation of equation 1, the more general equation 2 being obtained on largely similar principles (the full solution is given elsewhere: Conway, 1952).

DERIVATION OF THE EQUATION FOR THE ENERGY REQUIRED TO SEPARATE FLUID CONTAINING A SOLUTE FROM A LARGE AMOUNT OF MOTHER FLUID

A series of thermodynamically reversible and isothermal processes is considered which could be used to form a full

cycle and the original conditions restored, no energy being lost in the cycle. The solute is considered to be uncharged but the general equation is applicable to ions as noted above.

Stage 1. From the mother fluid there is expressed through a membrane a volume $V_f (= 1/C_{A_0})$ containing one mole of solute A, the membrane being permeable only to this solute. The work done on the system is:

$$\Delta W_1 = (\Sigma P_0 - P_{A_0}) \times V_f = RT\Sigma C_0/C_{A_0} - RT \qquad (5)$$

Here ΣP_0 is the total osmotic pressure of the mother fluid and P_{A_0} the osmotic pressure of the solute A therein, V_f being the expressed volume containing one mole of A, the concentration of A in this fluid being the same as in the mother fluid, so that

$$P_{A_0}V_f = RT$$

Stage 2. The volume V_f is now compressed reversibly (or expanded if $C_{A_1} < C_{A_0}$) by a piston with a membrane permeable only to water, until the volume V_1 is reached containing one mole of A in the required concentration C_{A_1}. The work done is given by:

$$\Delta W_2 = RT \int_{V_f}^{V_1} C_A dV = RT \ ln \ (C_{A_1}/C_{A_0}) \qquad (6)$$

Stage 3. The water separated in Stage 2 and in volume $(V_f - V_1)$ is allowed to enter the mother fluid, the reversible work done on the system being:

$$\begin{aligned} \Delta W_3 &= -\Sigma P_0(V_f - V_1) \\ &= -RT\Sigma C_0/C_{A_0} + RT\Sigma C_0/C_{A_1} \end{aligned} \qquad (7)$$

The total work required is then:

$$\begin{aligned} \Delta W_1 + \Delta W_2 + \Delta W_3 = \\ RT \ ln \ (C_{A_1}/C_{A_0}) - RT + RT\Sigma C_0/C_{A_1} \end{aligned} \qquad (8)$$

For one litre it may be written, as in equation 1:

$$\Delta W = RT[C_{A_1} \ ln \ (C_{A_1}/C_{A_0}) - C_{A_1} + \Sigma C_0] \qquad (9)$$

the general form of the equation being, as in equation 2:

$$\Delta W = RT[\Sigma C_{A_1} \ln (C_{A_1}/C_{A_0}) - \Sigma C_{A_1} + \Sigma C_0] \quad (10)$$

When the secretion is isosmotic with the mother fluid:

$$\Delta W = RT\Sigma C_{A_1} \ln (C_{A_1}/C_{A_0}) \quad (11)$$

or, as it may be written for the parietal secretion:

$$\Delta W = RT\Sigma C_p \ln (C_p/C_{pl}) \quad (12)$$

in which C_{pl} and C_p are the concentration in the plasma and the parietal fluid of any secreted ion or substance. Here it may be indicated that while this equation represents the work required to separate HCl hypothetically from the plasma, no such *separation* of free H^+ ions that were inside the cell occurs. None the less, however HCl is formed, it represents the work that could be theoretically obtained by the passage of such HCl *once formed* back into the plasma.

What is to be considered, therefore, is not the doing of work in the ordinary sense, but rather the *transference* of energy in one form associated with H atoms, into osmotic energy associated with H^+ ions.

COMPUTATION OF THE MINIMAL FREE ENERGY CHANGE INVOLVED IN THE PARIETAL SECRETION

The composition of the parietal secretion is taken from Gray (1943) along with Table VII. It follows from this table that for each mole of HCl secreted, 1532 0.159 = 9,600 calories are required.

This is similar to Hill's calculation of 9000 cals. (Hill, 1931: *Adventures in Biophysics*, p. 64.) In this he neglected the chloride change, as likely to require very little energy, and used equation 11. As in the next ex-

ample in that context, Hill uses the same equation for calculating the work done by the kidney, when secreting 2 per cent urea into the urine, where equation 10 would have been more appropriate (and in neither case would his more general equation 4, given above, have been valid) it would appear that he intended to give only very approximate calculation of energy requirements in secretion, without considering the exact formulation of the equations.

Many such calculations of the energy required for HCl secretion have been made (e.g., Borsook and Winegarder, 1931; Teorell, 1933; Hanke, 1937; Davenport and Fisher, 1940). Occasionally such calculations have been based on Hill's equation considered above, when the correct figure is not obtained.

TABLE VII

FREE ENERGY REQUIRED TO FORM 1 LITRE OF PARIETAL SECRETION

Ion	Plasma conc. (molar)	Parietal secretion conc. (molar)	Free energy change (cals.)
H^+	4×10^{-8}	0.159	−1,488.0
Cl^-	0.110	0.166	− 42.1
K^+	0.005	0.0074	− 1.8
Total			−1,531.9

The Source of the Secretion Energy

Under normal conditions the energy of secretion derives ultimately from carbohydrate. For the oxidation of glucose, as in the equation:

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O \qquad (13)$$

the free energy change at 38 °C. and under tissue conditions may be written:

$$\Delta G^\circ = -680{,}000 \text{ cals.}$$

This is but little different from the value of ΔG°, when all members of the equation are at unit activity.

Equation 13 might also be expressed:

$$C_6H_{12}O_6 + (6H_2O + 6O_2) \rightarrow 6CO_2 + 12H_2O \qquad (14)$$

to bring out the fact that throughout the aerobic metabolism of glucose six molecules of water enter at different stages and 24 atoms of hydrogen pass finally to oxygen. The overall oxidation may be divided as follows:

$$C_6H_{12}O_6 + 6H_2O \rightarrow 6CO_2 + 12H_2 \qquad (15)$$

$$12H_2 + 6O_2 \rightarrow 12H_2O \qquad (16)$$

For equation 15, $\Delta G^\circ = -6{,}277$ cals. (Wurmser, 1930) which is but a small fraction of the total. The value of $\Delta G^\circ = -679{,}680.$ cals. has been given for equation 16 as an independent reaction (Lewis, 1906).

The oxidation of glucose, from the energetic standpoint, is thus essentially an oxidation of hydrogen, and for each of the 24 H atoms passing to oxygen, a mean value of $\Delta G^\circ = -28{,}500$ cals.

If the oxidation of the metabolic hydrogen is considered to pass through H_2O_2, it may be divided into the stages:

$$24H^+ + 24\epsilon + 12O_2 \rightarrow 12H_2O_2 \text{ (aq)} \qquad (17)$$

$$\Delta G^\circ_{298} = -378{,}000 \text{ cals.}$$

and

$$12H_2O_2 \text{ (aq)} \rightarrow 12H_2O + 6O_2 \qquad (17a)$$

$$\Delta G^\circ = -301{,}000 \text{ cals.}$$

(calculated from the data of Lewis and Randall, 1923).

In recent years, owing to the failure to detect H_2O_2 in the metalloprotein transfer of electrons to oxygen, the reduction to H_2O has been assumed to occur, without pass-

ing through the intermediate stage of H_2O_2 and even a direct reduction of oxygen to OH^- has been represented. Seeing that H_2O_2 has been recognized in palladium and copper catalysis of the reduction of oxygen (Oppenheimer, 1939) and that iron is particularly active in catalysing the breakdown of the peroxide (apart from the specific activity of catalase which is in great abundance in cells) the position would appear at least doubtful. The representation of a reduction of oxygen to OH^- ions is objectionable, at pH values near the neutral point, since the hypothetical ions of atomic oxygen would pass almost entirely and practically instantaneously into water by the acceptance of H^+ ions. In this case oxygen cannot properly be held reduced to hydroxyl ions, but rather to water, as in the equation:

$$O_2 + 4\epsilon + 4H^+ \rightleftarrows 2H_2O \qquad (18)$$

the standard potential of which, indirectly determined, is about 1.23 volt, or 0.81 at pH 7. The oxygen pressure has a scarcely appreciable effect over a wide range, as it enters as the one-fourth power into the calculation.

Concerning the Expression of the Standard Free Energy Change in a Chemical Reaction

The well-known relation of this change to the equilibrium constant may be briefly considered, and a simple example chosen which could be suitably generalised.

If there is a reversible reaction such as:

$$A = B + C \qquad (19)$$

and $G^\circ_{298} = -1{,}000$ cals., this signifies that 1,000 calories of free energy are available in the change at 25 °C., of one mole of A into one of B and C, each in their standard states, or states of unit activity. Assuming any equilib-

rium relation for A, B and C with activities (A), (B) and (C) less than unit activity, and that one mole of A in the standard state is changed to (A), with $\Delta G = -RT\ ln(A)$, and then to B and C at (B) and (C) levels, there is no energy change in this latter transfer. After this it may be supposed that the moles of B and C which have originated in the chemical change are concentrated to the standard states of unit activities with $\Delta G = RT\ ln\ (B) + RT\ ln(C)$, the total energy change being:

$$\Delta G^\circ = -RT\ ln\ \frac{(A)}{(B) \times (C)} = -RT\ ln\ K \qquad (20)$$

(For unionized solutes in dilute solution, concentrations may be substituted for activities.)

In this derivation of ΔG° it may be noted that in the actual change of A to B and C no energy is liberated, but whatever the pathway, it still remains true that in the overall change of one mole of A in its standard state to B and C in their standard states, the energy change is $-RT\ ln\ K$.

If the change of A to B and C occurred at any activities other than standard values, say (A)′, (B)′ and (C)′, then from similar reasoning the change of free energy on the change of one mole of A may be written:

$$\Delta G = -RT\ ln\ K + RT\ ln\ \frac{(A)'}{(B)' \times (C)'} \qquad (21)$$

If $-RT\ ln\ K$ is numerically very large or K very small, observed concentrations (A)′, (B)′ and (C)′ will often make the value of the second expression on the right of the equation low compared with the first, and the observed change of energy similar to the standard change. Apart from such conditions these may differ considerably.

THE ENERGY OF THE GLUCOSE OXIDATION CONSIDERED AS ELECTRON CARRIAGE ACROSS REDOX POTENTIALS

The available energy may also be regarded from the standpoint of electron carriage across potential differences of redox systems.

The energy change is given by:

$$\Delta G = nF \times (E_h - E'_h) = 23{,}060 \times n \times (E_h - E'_h) \quad (22)$$

where n is the equivalent of electrons carried and F the Faraday constant. E_h, the potential of a system against the normal hydrogen electrode, is defined by:

$$E_h = E_0 + \frac{RT}{nF} \ln (Ox)/(Red) \quad (23)$$

where (Ox) and (Red) are the concentrations, or, more strictly, the activities, of the oxidised and reduced states of the substance involved in the process:

$$Ox + n\epsilon \rightleftarrows Red \quad (24)$$

If equation 23 is multiplied across by nF, it will be seen that there are two energy components, electrical and osmotic, indicating the manner in which the equation is derived, namely by the hypothetical carriage of unit quantity of electrons to or from a platinum electrode (an indifferent electrode regarded as an electron reservoir) to the solution at equilibrium with the electrode. The energy is thus made up of two components, electrical, or work done against the potential level (nFE type), and osmotic, or carriage from one to another level of concentration or chemical potential ($RT \ln a_1/a$ type). The total energy change is equated to zero, and so the form of the above equation is derived.

Equation 23 at about room temperature, and where one electron is involved may be written:

$$E_h = E_0 - 0.06 \log (Red)/(Ox) \quad (25)$$

or where two electrons are involved;

$$E_h = E_0 - 0.03 \log (Red)/(Ox) \quad (26)$$

From this equation it may be calculated that a difference of only − 30 millivolts is associated with a change of the oxidised form, from 90 to 10 per cent.

A rise to 180 millivolts above the characteristic potential with a two electron change will result in the oxidised form having one million times the concentration of the reduced.

In the oxidation of the 24 metabolic H atoms 24 electrons may be considered brought across the mean difference of potential between the dehydrogenase systems and the oxygen system:

$$O_2 + 4\epsilon + 4H^+ \rightleftarrows 2H_2O \quad (27)$$

the characteristic potential of which at pH of 7.0 is + 0.82 volt.

Assuming a passage of the 24 electrons from a mean E value of −0.3 volt (or near thereto) to + 0.82 volt the energy change is:

$$-24(0.82 + 0.3) \times 23{,}060 \text{ cals.} = -620{,}000 \text{ cals. (approx.)} \quad (28)$$

which would account for over 90 per cent of the total free energy change. The region of potential change from cytochrome c (+ 0.25 volt) to the oxygen system (+ 0.82 volt at pH = 7.0) may be regarded as unlikely to lead to potential energy transfers such as the H^+ ion accumulation of the gastric secretion, and phosphorylation is not known to occur within this region. Thus of the total energy liberated in the oxidation of glucose only a little

over half may be considered available, or for each gram molecule of oxygen about 60,000 cals. and if the energy required to excrete 1 gram molecule of HCl is about 10,000 cals. a Q_{HCl}/Q_{O_2} ratio of about 6.0 is to be expected as a probable maximum.

THE QUESTION OF THE SIGNIFICANT Q_{HCl}/Q_{O_2} VALUES FOR THE OXYNTIC CELLS

Much higher levels than 6.0 have been considered by Davies (1947), Crane and Davies (1948) and Davies and Ogston (1950) as experimentally significant. Ratios as high as 11 were occasionally found which signifies an efficiency of about 100 per cent. Further, "such a value may be maintained for a considerable period of time" (Davies and Ogston, 1950). Besides, values at or near this level appear to occur when the mucosa has inadequate carbon dioxide (Crane and Davies, 1948), a condition, according to Davies and Longmuir (1947), leading to ulcer formation. That an isolated tissue should perform its special function with about 100 per cent efficiency, and for a considerable time under difficulties, leads one to suspect that something is amiss with the calculation. It may well be in the assumption that only the *extra* oxygen after histamine is associated with the HCl production. The experiments for which exact quantitative data are available were carried out on frogs' gastric mucosae, and, as already noted, these appear to be in large part formed of oxyntic cells. There seems no adequate reason to suppose that the general carbohydrate metabolism in these cells (of which the oxygen consumed is an index) is not involved in the acidic process. From an inspection of the data of Davies (1948) it would appear that the maximum of the Q_{HCl}/Q_{CO_2} is about 2.0, where Q_{CO_2} represents the *total* CO_2 production being in turn a measure of O_2 uptake.

Though the quotient for the oxyntic cells may be somewhat more (depending on the proportion of their resting oxidation to that of the whole tissue), it may be only a little more.

With mammalian mucosae, where the parietal cells constitute a smaller fraction of the whole, even in their resting state they may use oxygen at a far higher level than the other cells of the glandular tubule or the surface epithelium.

SUMMARY

An equation is given for the reversible work done in separating a litre of fluid containing solutes with different concentrations from those of an unlimited volume of the mother fluid, and the derivation of the equation for the secretion of a single solute is described.

This equation differs from that of Hill (1914, 1924) which has a limitation not generally recognised.

When a secreted fluid is isosmotic with the blood, the equation has the form:

$$\Delta W = RT\Sigma C_{A_1} \ln (C_{A_1}/C_{A_0})$$

and is similar to that used by Gray (1943).

His estimate of the energy required in secreting a litre of parietal secretion at 38°C. is 1,532 cals. For one equivalent of H^+ ions it is approximately 9,600 cals.

The general source of the energy is discussed, and also the question of the real ratio of HCl secreted to oxygen consumed in this process by the parietal or oxyntic cells.

For a general treatment of energetics in biological activity, the reader is referred to such books as that of Bladergroen (1949) or of Clark (1948), and for the more formal treatment of chemical thermodynamics to such textbooks as those of Lewis and Randall (1923), Guggenheim (1933) or of Steiner (1941).

CHAPTER VIII

ENERGETICS (*Continued*)

A "Redox" Pump

REACTIONS LEADING TO THE LIBERATION OF H^+ IONS

(a) A metabolite is dehydrogenated and H atoms pass to co-dehydrogenase at a potential of about -0.3 volt. During this process free energy is trapped in 1.3-diphosphoglyceric acid. The acyl phosphate is an energy-rich phosphate bond and transfers phosphate to ADP.

(b) The further passage of the metabolic hydrogens is then either directly to a metal catalyst (or through an intermediate stage such as flavoprotein or a flavine carrier). Assuming a single valency change on the metal catalyst we may write:

$$RH_2 + Co \rightleftarrows C\bar{o}H + H^+ + R \qquad (1)$$

$$C\bar{o}H + 2M^{n+} \rightleftarrows Co + H^+ + 2M^{(n-1)+} \qquad (2)$$

or,

$$C\bar{o}H + H^+ + \epsilon + FP \rightleftarrows Co + FPH_2 \qquad (3)$$

and,

$$FPH_2 + 2M^{n+} \rightleftarrows FP + 2H^+ + 2M^{(n-1)+} \qquad (4)$$

where Co, FP and M^{n+} are the oxidised forms of cozymase, flavoprotein and metal catalyst.

It is with such or similar reactions that energy from the redox systems is transferred to the chemical potential of the H^+ ions secreted.

The "Redox Pump" as a Contribution to the Theory of the Biological Performance of Osmotic Work (Conway, 1951)

In the transfer of H atoms to the metal catalyst over a step in redox potential, osmotic as well as electrical work is done. It becomes of much interest to inquire how these are related, or what is the intimate nature of the change involved, which amounts to a utilisation of the primary energy of the foodstuffs, other than through the phosphate bond process.

In the elucidation of the immediate question it will be assumed that a redox system, $CtH_2 \rightleftarrows Ct + 2H$, transfers within the cell its H atoms to the metal catalyst system and that steady states result so that the relative Red and Ox proportions remain the same. At the pH of the cell then electrical and osmotic energy changes occur when H atoms are transferred and may be expressed as:

$$-\Delta G_1 = 2F(E'_M - E_{ct}) + 2\mu_c \qquad (5)$$

where the electrons of the H atoms are transferred across the potential difference of the systems, and 2 equivalents of H^+ ions are released at the chemical potential of the cellular H^+ ions.

It will be assumed then that instead of going directly through this path, the system $CtH_2 \rightleftarrows Ct + 2H$ operates in the acidic region, or simply that the molecules of CtH_2 enter the acidic region and their transfer of H atoms to the metal system in the outer region has the same overall rate as before.

With the transference of H atoms in the outer region we may write for the change in electrical and osmotic energy:

$$-\Delta G_2 = \xi + 2\mu_0 \qquad (6)$$

In the two paths H atoms may be regarded as passing

from the same initial state and splitting on the way into H ions and electrons, the latter likewise ending in the same system. It follows that:

$$2F(E'_M - E_{ct}) + 2\mu_c = \xi + 2\mu_0 \quad (7)$$

or,

$$F(E'_M - E_{ct}) - \xi/2 = \mu_0 - \mu_c = RT\ ln\ (H)_0/(H)_c \quad (8)$$

Here it may be questioned whether the electro-chemical potential of the H^+ ions should not have been represented above rather than the chemical. From the fact that Cl^- ions pass in practically equivalent amounts but only across a relatively slight concentration difference, the net result would be very similar. Also, with yeast the high levels of $(H)_0$ described are produced by exchange of H^+ ions for K^+ ions and so negative the effect of the potential across the membrane. Here also the passage of the balancing ion under these conditions (with high external K^+ ion concentration) is over a relatively small change of chemical potential and does not appreciably affect the significance of equation 8.

It will be seen then from equation 8 that as the osmotic energy increases the electrical energy in the outer region decreases to the same extent. The maximum transference is obtained when ξ is zero, and:

$$F(E'_M - E'_{ct}) = RT\ ln\ (H)_0/(H)_c \quad (9)$$

It will also appear that such could only occur when the rise in chemical potential of the H^+ ions causes a corresponding rise in the redox potential of the H atom transferring system. This property, however, is common to all H atom transferring redox systems, but not to the metal redox systems transferring only electrons. From equation 9 the essential step in redox potential between the operating systems may be calculated.

Thus if for the parietal cell $(H)_0$ is approximately 10^{-1} and $(H)_e$ is approximately $10^{-6.4}$ then the value of $(E'_m - E'_{ct})$ at this pH and 18°C. should be about 0.33 volt. For the observations on yeast, similar calculations at about 18°C. give the $(E'_m - E'_{ct})$ value as approximately 0.25 to 0.30 volt. It is not essential that the E values are the same as the characteristic potentials of the systems at the pH of the cell, and therefore the process could be worked with a much smaller step in the characteristic potential levels, the metal system being in large part oxidised and the H donor reduced.

Table VIII shows the characteristic potentials of a number of metal catalyst and possible H donors.

Generalisation of the Above View

It will be seen that the above process of converting electrical energy to osmotic need not be confined to the H^+ ions. A possible generalisation may be attempted as follows.

An electron donor system is taken for illustration, rather than a H atom donor system, though this latter can also be included. A possible mechanism for the active and direct secretion of an inorganic cation may be selected as an example.

Accompanying Cl^- ions may be considered as passively carried by the establishment of a potential difference (such as Ussing investigated for the frog skin, 1948).

The appearance of electrons from metabolic H atoms and their disappearance on final transfer to oxygen, may be considered operative in the same cell region or side of the membrane, or equally operative in both, so that the pH need not change.

At the same time it is assumed that a metal electron carrier system is irreciprocally fixed in the membrane, so

that it can neither diffuse from its position nor act on alternate sides by rotation. A complex more or less specific with the cation and a metal catalyst is then con-

TABLE VIII

STANDARD POTENTIALS OF SOME METAL CATALYSTS AND POSSIBLE H DONORS

(From the list compiled by Anderson and Plant, 1949)

System	Temp. (° C.)	pH	E'_0 (volts)
Acetaldehyde/acetate	...	7.0	−0.468 (calc.)
Glucose/gluconate	...	7.0	−0.45
Isocitrate/oxalosuccinate	...	7.0	−0.30
Co-enzyme I	30	7.0	−0.28
Glyceraldehyde - 3 - phos./1,3 - diphosphoglycerate	...	7.0	−0.28
Various isoalloxazines	20	7.0	−0.207 to −0.012
Lumiflavin	20	7.0	−0.207
Riboflavin	20	7.0	−0.186
Riboflavin phosphate	20	7.0	−0.185
Lactate/pyruvate	35	7.0	−0.180
Ethanol/acetaldehyde	25	7.0	−0.163 (calc.)
Cysteine/cystine	25	7.0	−0.14
Thioglycollic acid	25	7.0	−0.14
Malate/oxalacetate	38	7.0	−0.102
Yellow enzyme	38	7.0	−0.060
Succinate/fumarate	...	7.0	0.00
Glutathione	25	7.0	+0.04
Heme, ferro/ferri	30	8.18	−0.188
Cytochrome b	20	7.4	−0.040
Cuprous/cupric	25	0.0	+0.167
Cytochrome c	...	7.0	+0.262
Cytochrome a	20	7.4	+0.29
Ferrous/ferric	25	0.0	+0.771
Water/oxygen	25	7.0	+0.815

sidered to form within the cell when the reduced catalyst exposes a negative charge on its total enzyme complex.

This acts then in the outer region passing its electron to the fixed metal catalyst and liberating Na^+ ions. The electron moves from the fixed catalyst in the membrane to the

third (which can operate across the membrane) and finally to oxygen within the cell. As the electron is carried back into the cell it exchanges with an anion to maintain the electrical balance. It will be seen that such a process is similar in essential features to the HCl secretion by the parietal cells of the gastric mucosa, but the H^+ ion concentration does not alter. As before, electrical energy is converted into osmotic.

Such a scheme may be presented in a variety of ways and it is of interest to note that the upper reach of redox potentials, not directly associated with formation of phosphate bond energy, could possibly be utilised in this way.

It is also attractive for the reason that the active carrier and the immediate energy source are one and the same system.

With regard to the specificity of carriers for inorganic cations, the relatively high specificity of the active absorption of K^+ ions in fermenting yeast is an example, and for Na^+ ions one may cite the slow active extrusion of Na^+ ions from the muscle fibre (Conway and Hingerty, 1946) and the specific active carriage of Na^+ ions across the frog's skin as examined by Krogh (1937) and Ussing (1948).

Another possible mode of secretion of an inorganic cation like the Na^+ ion is, as a secondary process after primary H^+ ion secretion, by exchange with H^+ ions. This has been advanced by Davies and Ogston (1950).

Such exchange after H^+ ion secretion has long been considered by Teorell in his "diffusion theory" (1933, 1939) and there is no doubt that the process occurs, but the exchange can scarcely be regarded as a direct functional secretion of Na^+ ions. Under any condition in which a high Na^+ ion concentration is secreted in exchange for H^+ ions, there must be an initial high level of $[H^+]$, some-

thing, in short, like what happens under gastric conditions. The more direct type of inorganic cation secretion outlined above is in better accord with the idea of a functional secretion of Na^+ ions.

The Passage of the Electrons into the Interior of the Parietal Cell after Liberation of H^+ Ions

When the reduced metal catalyst operates in the interior of the cell, either by actual diffusion through the separating membrane or by rotation therein due to thermal agitation (see Lundegårdh, 1946, 1947) or simply by electron transference in exchange for Cl^- (or succinate ion) it is only necessary for the working of the acidic mechanism that the electrons be transferred to some accepting system. The potential of this may differ only a small amount from that of the donator system, the minimal amount being governed by kinetic considerations.

Utilisation of the Surplus Energy of the Electrons after the Splitting of the Metabolic H Atoms into H^+ Ions and Electrons

By the surplus energy is here meant the energy involved in the electron transit from the redox potential of the metal catalyst in the acidic region to the oxygen system.

From the potential level of cytochrome c ($E'_0 = +0.25$ volt) to that of the oxygen system ($E'_0 = +0.82$ volt), ΔG for the 24 metabolic H atoms has the value of $-310{,}000$ cals. Within this zone of potential no phosphorylation has been recorded, and as previously noted there is little likelihood of its being utilised for the acidic mechanism, apart from its acceptance of electrons therefrom. Thus 370,000 cals. or 62,000 cals. per mole of oxygen, may be considered available with approximately 10,000

cals. required for each equivalent of H ions, giving a maximum Q_{HCl}/Q_{O_2} value of about 6.2.

Apart from their experimental results Davies and Ogston (1950) get a theoretical total value of 10.8 for the processes they have considered. This would necessarily imply a practically complete utilisation of the energy in the transfer from +0.25 volt to +0.82 volt, though no indication is given as to the manner in which this is accomplished.

Examination of their account shows, however, that in what they term mechanism I (corresponding to a scheme previously outlined, Conway and Brady, 1948) when metabolic H atoms are transferred to a metal system with formation of H^+ ions and retention of electrons, the potential energy of the H^+ ion concentration so generated is not referred to the immediate interaction of the systems involved, but rather to the further and complete path of the electrons across the cytochrome system to oxygen. Such treatment of the energy question is inadmissible and accounts for their high theoretical ratio.

The surplus energy that could probably be utilised in the gastric mucosa might lie between the potential levels of 0.00 and 0.25 volt or the region between the potential of the operating metal catalyst and cytochrome *c*. (In yeast this region could be set at a lower level.) For the scheme in yeast acting anaerobically it was indicated that an organic acceptor, R_1, received H^+ ions and electrons in the interior of the cell from the special metal catalyst and if this occurred in some measure in the animal cell the acceptor might be converted to a metabolite feeding H atoms again into the acidic system (Conway, 1949).

The change from the potential level of the metal catalyst to that of such metabolite would doubtless involve the use of phosphate bond energy. This process if it oc-

curred (and it would not appear necessary in order to explain the true ratios observed) could be thought of as involving circulation of the same electrons, (or, at least, the same quantity of electrons) derived originally from metabolites, with H^+ ions taken from those in the cell but provided in a steady state from the reaction:

$$CO_2 + H_2O \rightarrow H_2CO_3 \rightarrow H^+ + HCO_3^- \qquad (10)$$

The H^+ ions join with the electrons to form H atoms on the organic acceptor, which directly, or through an intermediate stage, returns these H atoms into the acidic system to form again H^+ ions and electrons.

All this would probably add only an additional 2.5 to the maximum Q_{HCl}/Q_{O_2} ratio of 4.0 for metabolic H atoms directly utilised.

A similar concept to the above has been put forward by Davies and Ogston (1950) and is described in Chapter VII.

THE PROCESS IN THE YEAST CELL

For the reaction:

$$C_6H_{12}O_6 \rightarrow 2CO_2 + 2C_2H_5OH \qquad (11)$$

$$\Delta G^\circ = -55{,}000 \text{ cals.}$$

For each molecule of glucose metabolised anaerobically four metabolic H atoms are released by dehydrogenase. The theoretical maximum for the ratio acid/alcohol is 2.0 but the observed ratio is very much less and in the region of 0.12. Only a small fraction of the metabolic H atoms are thus trapped and excreted as H^+ ions, and the energy change is much higher than that required for this secretion.

In the acidic process a fraction of the metabolic H atoms are diverted to the metal catalyst in the acidic region and from the fact that the maximum value of $RT\ ln\ (H_0)/(H)_c$

is about 6,000 cals., a potential difference of about 0.26 volt between the system feeding H atoms to the acid forming catalyst would be required. The E'_0 value for the acetaldehyde-alcohol reaction has had various values assigned to it: thus, −0.063 volt by Lehmann (1934); Barron (1939) calculates −0.190 volt from Adler's data (1937); Kalckar (1941) calculates −0.163 volt. (In a previous communication (1949) the author used the E_0 value in error for the E'_0, but without significance for the general context.)

SUMMARY

The energy changes involved in the redox cycle are considered in more detail.

In the scheme, for every mole of oxygen used by the oxyntic cells, four moles of HCl could be produced, but if the metabolic H atoms are again returned to the H^+ ion production system with use of phosphate bond energy, the ratio could be increased by about 2.2, to a total of 6.2.

The step in redox potential between the H atom feeding system and the metal catalyst liberating the H^+ ions is calculated. The principle used is made the basis of a contribution (the "redox pump" theory) to the general question of the manner in which the cell performs osmotic work.

CHAPTER IX

GASTRIC POTENTIALS

Gastric potentials were first recorded by Donné (1834) and their possible relation to the secretion of HCl has been long considered. In recent years the question has again been investigated by a number of workers (Swyngedauw, 1928; Mislowitzer and Silver, 1932; Sarre, 1934; Quigley, Barcroft, Adair and Goodman, 1937; Rehm and Hokin, 1947; Rice and Ross, 1947; Crane, Davies and Longmuir, 1948; Teorell and Wersall, 1945; Davies and Ogston, 1950). Using pouch dogs Quigley, Barcroft, Adair and Goodman (1937), considered that there was no relationship between the gastric potential and secretion, and other workers have provided a variety of conflicting reports.

In connection with such potentials it has to be borne in mind that they are not like potentials across the muscle fibre membrane, which would represent potential differences from within the cell or fibre outwards, but are potentials from one side of a cell layer to the other side, and thus correspond to some asymmetry of metabolism or structure.

The following is a summary of the chief facts now established, and is largely taken from the account by Rehm (1950) who has added much to our knowledge in this field. (His arrangement for observing gastric potentials *in situ* in the mammal is shown in Figure 23.)

(a) In general it has been found that potential differ-

ences exist across the stomach wall with the mucosa either resting or secreting, the serosa being positive in the external circuit to the mucosa.

(b) For the dog stomach with 0.9 per cent saline in contact with both mucosal and serosal surfaces, the potential difference is usually −70 millivolts, and that of the secreting stomach about −40 millivolts.

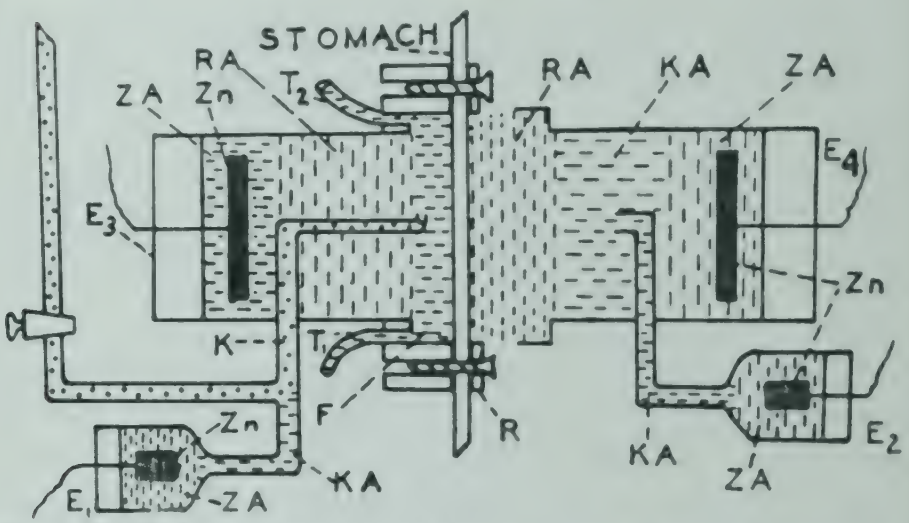

Fig. 23. Rehm's arrangement for measuring gastric potentials. Schematic drawing of a sagittal section of the apparatus with stomach in place. R refers to a lucite ring. This ring has an oblique cut in it so that the blood vessels (not shown) supplying the portion of the stomach in the chambers can be slipped inside it. RA refers to Ringer agar or saline agar; KA to saturated KCl agar; K to saturated KCl; F to the fluid in the chamber. Electrodes E_3 and E_4 were used for sending current through the stomach. (Courtesy of *Gastroenterology*.)

The decrease of potential differences with the origin of gastric secretion has been observed by Rehm (1944, 1946), Crane, Davies and Longmuir (1948), Mislowitzer and Silver (1932) and Rice and Ross (1947) who review the literature.

Crane, Davies and Longmuir found that the steady potential difference for the isolated gastric mucosa of the

frog varied between −7 and −50 millivolts, with a mean value of −30 millivolts (standard deviation: −9 millivolts). When the mucosa is first set up the mean value is about −20, changing to −30 millivolts after about half an hour. They suggest a dying away of injury potentials as the cause of the change. When stimulated to secrete, the magnitude of the negative potential decreases, the decrease being usually greater for higher rates of secretion than for low ones. They are of the order of 10 millivolts for a Q_{HCl} increase of 2, or of 17 millivolts for a Q_{HCl} increase of 6.

For the dog's stomach, Rehm (1944) and Rehm and Hokin (1947) find that, except at very low secretory rates, the potential difference of the secreting stomach shows very little variation during relatively large variations in the rate of secretion of H^+ ions.

(c) Rehm (1945) showed that the sending of current from an external battery from the serosa to the mucosa in a secreting stomach results in an increase in the production of H^+ ions, and the application of current in the opposite direction results in a decreased production. The application of current does not initiate secretion.

The effect on the secretion was confirmed by Crane *et al.* (1948) for the isolated mucosa of the frog.

ENERGY DERIVABLE FROM THE GASTRIC POTENTIALS

It has been found (Rehm, 1943; Crane, *et al.*, 1948) that when shunted through a low external resistance, the electromotive forces of the stomach can produce electric currents of some magnitude. The energy produced, however, under these conditions appears to be only a fraction of the minimal energy necessary for the production of HCl in the gastric secretion.

The possibility is discussed by Rehm (1950) that there may be electromotive forces in the mucosa not oriented so as to contribute materially to the potential difference across the wall and that these electromotive forces can deliver much more electrical energy than those controlling the potential differences observed, and produce the main energy for HCl production.

This is considered improbable chiefly for the following reasons:

1. A marked decrease in the potential difference observed was always associated with a marked decrease in the secretory rate.

2. This is so even when the observed potential difference is depressed by applied current, which could be expected to affect selectively the potential difference across the stomach wall.

It is concluded (Rehm, 1950) that the electrical energy produced by the stomach can probably furnish only a small portion of the energy necessary for the production of HCl.

Rehm also shows that the minimum free energy necessary to produce the increased H^+ ions resulting from the flow of current (from an external battery) from the serosa to the mucosa is greater than the amount of the applied electrical energy capable of doing useful work.

INTERPRETATIONS OF THE FUNCTION AND ORIGIN OF THE GASTRIC ELECTROMOTIVE FORCES GIVING RISE TO THE POTENTIAL DIFFERENCES ACROSS THE MUCOSA

It will be seen from the above that it is improbable that appreciable energy can be directly supplied in this way for the secretion of HCl, and those who in recent years entertained such a possibility or used it as an integral part of

their theoretical interpretation of HCl production appear to have now abandoned it.

At the same time Rehm (1950) puts forward the view that such electromotive forces may act as controls of the secretion of H^+ ions once these are produced, and that while only a small fraction of the secretory energy is available, yet enough coulombs of electricity can be produced for the carriage of the H^+ ions. "The return flow of current would be in the opposite direction and result in the transport of Cl^- ions from the interstitial fluid to the gastric juice."

Further, if the H^+ and Cl^- ions are thus secreted in slightly hypertonic concentration, they will in turn result in the passage of water across the cells (a view also advanced by the author, 1949a).

While this *controlling* function of the H^+ ion secretion may very well occur, nothing is indicated thereby as to the actual origin of the potential differences and it may be thought that these, unimportant from an energetic standpoint, may be only derivative phenomena of the secretory mechanism.

Davies and Ogston (1950) have attempted an interpretation of the origins of the gastric potentials from a study of electrode measurements within the frog's gastric mucosa.

The resting mucosa they find to be impermeable to Cl^- and H^+ ions. This conclusion is based on the observation that with the silver-silver chloride electrodes on the one hand, or glass electrodes on the other, placed in the secretory and nutrient fluid sides of the mucosa, that the potential differences recorded are expressed by:

$$x = \frac{RT}{F} \ln \frac{[Cl^-]_n}{[Cl^-]_s} - 0.03 \text{ volts} \qquad (16)$$

for chloride and a similar equation for the H^+ ion con-

centrations, using glass electrodes. Here −0.03 volt is the constant asymmetry potential (observed by calomel electrodes).

It is considered that if the mucosa were permeable to Cl^- ions, for instance, the first member of the above equation would vanish. It may be presented that in the case of the three ions, Na^+, H^+ and Cl^- (assuming such only to be present on each side of the mucosa) if only Cl^- ions were diffusible, then this would give rise to two diffusion potentials on each side with numerical values expressed again by the first member of equation 5 but with opposite signs. If both Na^+ and Cl^- ions were diffusible, then Donnan membrane potentials would result of the same magnitude, and cancel out in the same way. If the three ions were diffusible and originally different in concentration, no equilibrium could result.

Davies and Ogston then consider that, for the resting mucosa, if the Na^+ ion concentration is changed, the effect is not at all comparable to that with changes in Cl^- ions and pH. There is, however, some effect. It is concluded that the mucosa is permeable to Na^+ ions, and other evidence is cited in support (such as Visscher's observations with cationic dyes, 1942, which passed through the resting parietal cells). They proceed to represent the membrane as equivalent to a pair of reversible sodium electrodes and if at equilibrium under the conditions described "no difference of potential could arise between the pair of sodium electrodes." (This is so if there are the same Na^+ ion concentrations on each side of the membrane, but not if such are different and the supposed pair of sodium electrodes is the membrane itself.) It is then considered that an equilibrium condition does not in fact arise, but an exchange of Na^+ ions for H^+ ions leaking back or formed by the secretory mechanism and that the ap-

proach to equilibrium of this interchange is facilitated by a negative potential on the secretory side.

This, when introduced with respect to the resting gastric potential, appears to imply that the secretory mechanism is supposedly active in some degree in the resting stage.

In other words the resting gastric potential is considered to arise as a diffusion potential without equilibrium being attained. During secretion, this diffusion potential is lessened "by removal of HCl from the interior of the cell." Further clarification is here desirable.

Their conclusions in any case appear to have little value with respect to events in the oxyntic cells because:

(a) The observed mucosal or epithelial barrier contains three different kinds of cells (Fig. 5).

(b) Observed potentials may be related more to active transport th anpassive diffusion.

WHY DOES HCl NOT DIFFUSE BACK RAPIDLY INTO THE SECRETING CELLS?

In this we may include the yeast cell with the parietal cell and the question is very pertinent for the following reasons. During yeast fermentation in the presence of KCl and a rising acidity outside, a rapid entrance of K^+ ions into the cell can be observed. Also succinate ions come out rapidly from the cell. Then considering ion size why should not H^+ and Cl^- ions enter the cell with a rapidity at least equal to that of K^+ and succinate ions? This could not be explained by a mere difference of potential, including redox potentials, or by a flow operating against back diffusion but an answer may be provided as follows, and in agreement with the discussion in previous chapters.

The inner membrane of the "acidic" region in yeast is *relatively* impermeable to free anions and cations at the beginning, at least, of the acidic formation. A *slow* pas-

sive exchange of K^+ ions for the cellular potassium can in fact be observed in resting yeast (Conway and Moore, 1950).

The selective permeability of K^+ ions and succinate ions is then explained by specific adsorptions on carrier molecules. Here K^+ ions may be said to displace H^+ ions at such a surface and are preferentially withdrawn into the cell. (The degree of displacement will relate to the relative concentrations; Conway and O'Malley, 1946.) The carrier molecules are such as previously considered, namely a heavy metal catalyst which undergoes a valency change in oxidation and reduction and releases or acquires electrons, in a redox cycle.

In the absence of added K^+ ions, H^+ ions are formed from the ionization of the reduced metal catalyst and excreted in association with succinate ions, as succinic acid. The overall electrical exchange is one of electrons for succinate ions. It may be possible to explain the occurrence by an electron gradient producing a potential difference and a current across the membrane. The return current would carry the succinate ions which would not otherwise diffuse out. However this may be, the fact that when K^+ ions are present, their preferential carriage across the membrane can scarcely be explained without recourse to specific adsorption and the displacement of H^+ ions at a surface.

Concerning the parietal cell, with secretion strongly acidic, the fact that HCl does not return to the cell or that it moves practically in only one direction, is explicable only if the H^+ ions are formed from the redox catalyst on the *secretory side of a cell membrane* and that this membrane is relatively impermeable to cations.

With regard to the Cl^- ions, specific adsorption with carriage is improbable, and it would seem more satisfactory

to consider the membrane permeable to Cl^- ions, under these conditions the Cl^- ions being carried outwards by a current, somewhat in the manner described by Rehm (1950).

SUMMARY

It has been shown by Rehm (1950) that the gastric potentials are quite inadequate for supplying the energy necessary for the HCl secretion, though they may exercise a controlling function.

On the other hand they may be only a consequence of the operation of the redox system across the membrane.

The question why HCl does not rapidly return to the secreting cell is briefly considered.

CHAPTER X

NEUTRALISATION, ALKALINE AND ACIDIC

THE HIGH acidity of the parietal secretion is associated with an equivalent alkalinisation within the secreting cells. A restoration of H^+ ions to the cells becomes essential. Likewise the non-parietal cells, chiefly as it would seem, those secreting mucus, require protection from the acidity of the gastric juice.

Two intracellular enzymes have been shown to catalyse neutralising reactions. These are carbonic anhydrase and urease. Carbonic anhydrase catalyses the restoration of H^+ ions to the oxyntic cell and urease the formation of ammonia, protecting the non-parietal cells from excess acidity. The urease-ammonia mechanism (FitzGerald, *et al.*, 1946–1951) is possibly the most significant of the acidic neutralising processes as considered in this and the following chapter.

THE ROLE OF THE CARBONIC ACID SYSTEM IN THE PRODUCTION OF HCl

The process of restoring H^+ ions to the cell occurs through the following reactions:

$$RH \rightarrow R + H^+ \qquad (1)$$

$$RNH_3 \rightarrow RNH_2 + H^+ \qquad (1a)$$

and

$$CO_2 + H_2O \rightarrow H_2CO_3 \quad (2)$$

$$H_2CO_3 \rightarrow H^+ + HCO_3^- \quad (3)$$

Equations (1) and (1a) represent the supply from the fixed buffers, proteins, esters of phosphoric acid, etc., and equations 2 and 3 represent the supply from carbon dioxide. Probably only a very small amount comes from reactions 1 and 1a and at the outset of the HCl secretion it will be equal to the increase of pH in the cell fluid multiplied by the buffering.

When a steady state occurs all the H^+ ions are being supplied by reactions 2 and 3, and as HCO_3^- ions increase they exchange for external Cl^- ions to establish the Donnan relation between cell and intercellular fluid:

$$(Cl^-)_i/(Cl^-)_c = (HCO_3^-)_i/(HCO_3^-)_c \quad (4)$$

(corresponding to the so-called Cl^- ion shift in the red corpuscle).

This effect of CO_2 and the exchange of HCO_3^- ions with Cl^- ions was described by Davenport (1943).

At slow rates of HCl secretion it may be supposed that the metabolic production of CO_2 suffices for the restoration of H^+ ions. In such metabolic production there is also some evidence to show that the primary product may be carbonic acid or its ion rather than CO_2 (Conway and O'Malley, 1948) though the path now appears to depend on the conditions (Conway and O'Malley, 1951). At high and normal rates of acid secretion, the HCl formation exceeds the metabolic production of CO_2 (Davies, 1948) and a considerable fraction must be supplied from the blood.

In a steady state as many HCO_3^- ions pass into the blood as Cl^- ions or HCl into the stomach. Davenport discussed the question whether CO_2 could furnish HCO_3^- ions with sufficient speed, without being catalysed (1946). With data for the rat the uncatalysed reaction 2 (above)

would appear to yield HCO_3^- ions at the required order of rate for the HCl secretion. He considered, however, that more refined experiments might demonstrate the need of carbonic anhydrase, which he had shown to be present in very high concentration.

In subsequent calculations Davies and Roughton (1948) refer to Davenport as neglecting the back reaction (CO_2 from HCO_3^- ions) and they use much higher figures for the HCl secretion (per unit weight of mucosa) based on cat and human data. They conclude that catalysis is necessary. While the data for cat and man lead to this conclusion, it may be said that their calculations with respect to the back reaction are somewhat arbitrary, as will appear from the following.

The rate of disappearance of CO_2 may be represented by the equation:

$$d[CO_2]/dt = -k_1[CO_2] + k_2(H_2CO_3) = -k_1[CO_2] + k_2\frac{(H^+)(HCO_3)}{K_1} \quad (6)$$

(neglecting the small degree of ion catalysis, Roughton, 1943) where (H^+) and (HCO_3^-) are the activities of the H^+ and HCO_3^- ions and k_1 the velocity constant the reaction:

$$CO_2 + H_2O \rightarrow H_2CO_3 \quad (7)$$

and k_2 that of:

$$H_2CO_3 \rightarrow CO_2 + H_2O \quad (8)$$

K_1 being the ionization constant at 38 °C. of the reaction:

$$H^+ + HCO_3^- \rightleftarrows H_2CO_3 \quad (9)$$

The activity of HCO_3 is taken as $0.66 \times [HCO_3^-]$ at an ionic strength of 0.13 (Hastings and Sendroy, 1925).

Roughton gives values of k_1 = 89 moles/l./sec., k_2 = 0.131 mole/l./sec. and pK = 3.47 at 38 °C. and at infinite dilution.

For $[HCO_3^-]$ they use a figure of Irving (1932) for the total CO_2 content of the dog mucosa (fundus region). Since the oxyntic cells form only about 10 per cent of the whole mucosa this figure here has no significance.

Of greater consequence is the implied assumption that $[HCO_3^-]$ would remain constant, when the secretion begins. The value of the second component in equation 6 depends on the product $(H^+)(HCO_3^-)$ and with the removal of H^+ ions from the cell and the very probable decrease of Cl^- ions within the cell, owing to the flow outward into the lumen it is not unlikely that both (H^+) and (HCO_3^-) may fall and the value of the second component in equation 6 becomes relatively very small. In short, attempts to calculate the back reaction have at present little value, as it depends much on factors very difficult to determine, and for which there are no adequate data. Such would include permeability rates, diffusion distances and metabolic levels in the parietal cells.

All that can be reliably said is that the *maximum* rate of change of CO_2 is given by $-k_1[CO_2]$ which accords with Davenport's initial approach.

From Roughton's figure for k_1 and taking the free CO_2 as 1.0×10^{-3} or near thereto, this upper limit of the rate of CO_2 disappearance is 1.3×10^{-4}/moles/l./sec., or approximately 0.5 mole/l./hour. At 25 °C. for the frog with a free CO_2 figure of about 0.8×10^{-3} moles/l. and k_1 = approximately -0.4×10^{-4}/moles/l./sec. it is 0.12 mole/l./hour.

Table IX gives the order of figure for the ratios of HCl excreted or HCO_3^- ions formed to the maximum rate of the uncatalysed disappearance of CO_2.

It will be seen that for man, cat and dog catalysis is necessary, as the HCl is secreted at a rate considerably above the maximum non-catalysed rate. Possibly also for the frog and rat, if the true value of the back-reaction

TABLE IX

HCl Secretion Rates and Maximum Rates of CO_2 Disappearance

Animal	Wet weight of mucosa in g.	Oxyntic cells as fraction of mucosa	Average secretion of HCl after stimulation (mmoles/hour)	Average secretion of HCl after stimulation per litre of oxyntic cells (moles/hour)	Ratio of secretion rate per litre oxyntic cell to max. rate of uncatalysed disappearance of CO_2
Rat	0.5	0.13	0.05	0.77	1.6
Man	60	0.10	15	2.5	5
Dog	2.8 (amount examined)	(0.10)	2.6	9.8	20
Cat	13.5	0.07	5.6	5.9	12
Frog	0.10	0.25 (?)	0.0045	0.18	1.5

The data for the rat are from Davenport (1946). For man the average weight of the mucosa as well as the oxyntic cell fraction are from Davies (1948). The secretion rate is from Ihre's data (1943) in which errors from saliva and duodenal regurgitation are excluded. Data for a dog are taken from Rehm and Enelow (1945). The cat data are for one cat for which the various figures were obtained by Mackay and Davies (1948). Frog data in 2nd and 5th cols. are judged from results of Davies (1948).

could be computed, and the above data more precisely determined. This is scarcely surprising as the parietal cell contains far more carbonic anhydrase than the red corpuscle (Davenport, 1939; Davenport and Fisher, 1940).

DAVENPORT'S DISCOVERY OF LARGE AMOUNTS OF CARBONIC ANHYDRASE IN THE PARIETAL CELL

Davenport and Fisher (1940) found much carbonic anhydrase in the gastric mucosae of cats, rats and rabbits

(Behrend, 1937, had previously failed to obtain appreciable amounts). In the following year Davenport set about investigating the cells on which the enzyme was located. Cats and rats were used. The blood in the viscera and gastric mucosa was removed by a perfusion with 0.6 per cent saline containing 6 per cent gum arabic. The carbonic anhydrase was determined by the method of Meldrum and Roughton (1933).

Investigating the distribution of the enzyme, cylinders were stamped from the gastric mucosa by means of a cork borer, and the enzyme determined therein. The general distribution in the mucosa followed that of the parietal cells.

A modification of the method of Glick *et al.* (1935) was used to determine in which cells the enzyme was present. From cylinders, 4 mm. diameter, stamped from the frozen mucosa, sections were cut in a freezing microtome perpendicular to the gland lumina. Slices 0.020 mm. were cut and groups of three consecutive satisfactory sections were then examined, the groups being distributed at random along the cylinders. The first and third sections were examined for carbonic anhydrase, and the middle section fixed, stained and the parietal and other cells counted. The correlation between enzyme concentration and the number of parietal cells had the very high value of +0.95.

Figure 24 shows the graph of number of parietal cells and the carbonic anhydrase in enzyme units (Davenport units = $(R_1 - R_2)/R_1$ where R_1 and R_2 are the reciprocals of the time, without and with the enzyme, for the evolution of a certain definite amount of CO_2 under standard conditions).

Such results could be simulated by some other cell type if this had an almost perfect correlation with the parietal

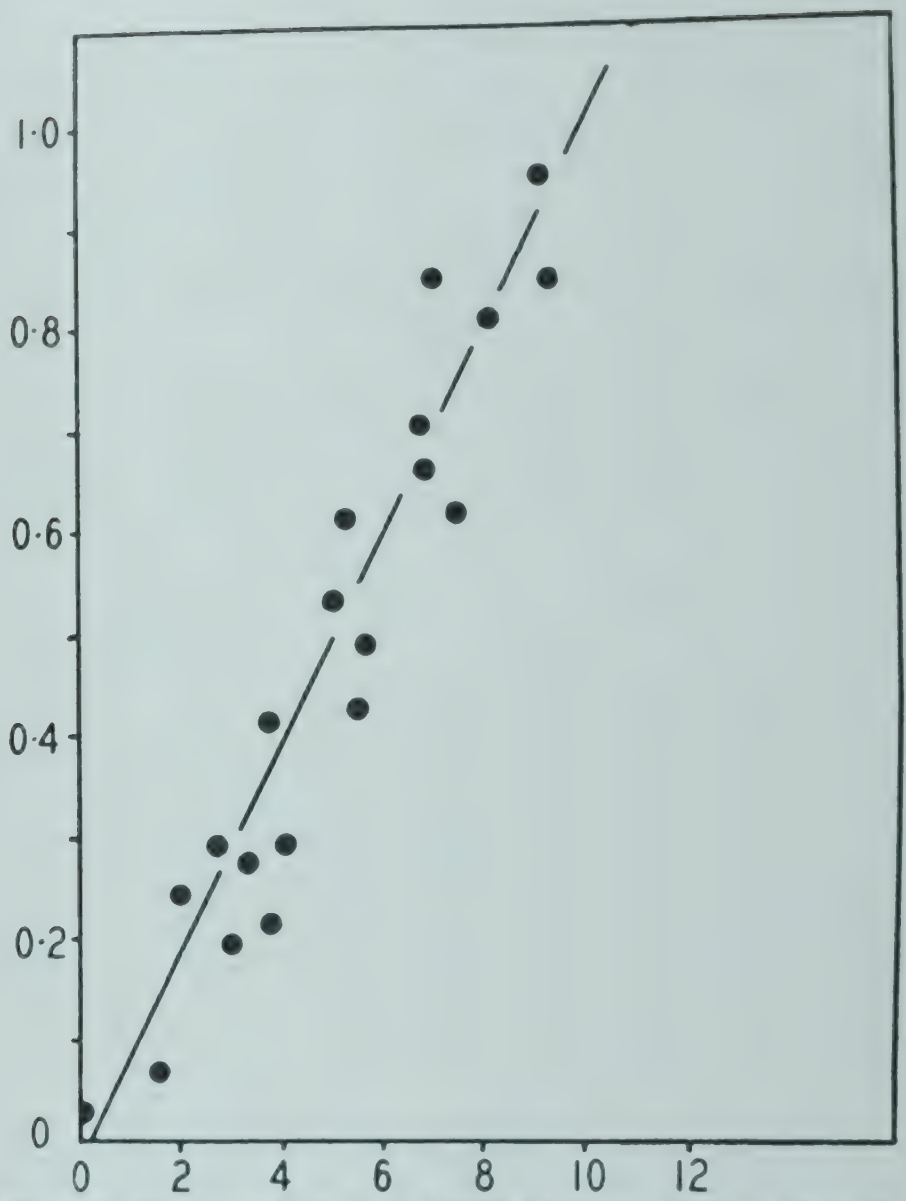

Fig. 24. Davenport's diagram (1939) Abcissae: 1000 parietal cells per slice gastric mucosa of the cat; ordinate: carbonic anhydrase in Davenport units per slice. (Courtesy of *Am. J. of Physiol.*)

cell, over counts extending from zero up to about 10,000, but as there is no evidence of such, it may be concluded that the parietal cells contain the carbonic anhydrase.

Davenport found the surface cells to contain about one-tenth the amount of enzyme as the parietal cells.

For the cat the concentration of enzyme in the parietal cell was found to be about five to six times that in the red corpuscle, and for the rat about three times. Similar high values were obtained for the parietal cells of the dog mucosa, which would appear to contain roughly five times the amount in the red corpuscles (Davenport, 1939 to 1940).

Effects of Inhibiting the Enzyme

The very high concentrations of carbonic anhydrase in the oxyntic cells appeared to Davenport to signify some direct relation of carbonic acid to the excretion of HCl. This view was withdrawn (1946) as the result of a study of the effect of carbonic anhydrase inhibitors on gastric secretion, though, as he pointed out later (1946), the inhibition was not sufficiently complete in such experiments to warrant the conclusions drawn from them, the concentration of the enzyme being so great that even a 99.9 per cent inhibition would still leave very appreciable activity.

The present position is that the evidence now supports the view that the enzyme is necessary to catalyse the restoration of H^+ ions to the cell via carbon dioxide and $HC\bar{O}_3$ or that the enzyme and the carbonic acid system have an indirect effect on the HCl output by maintaining the necessary conditions for the sustained secretion.

NEUTRALISATION OF ACID IN THE PARIETAL SECRETION

Various ways in which the concentration of acid in the parietal secretion or in the gastric juice may be decreased can be listed as follows:

1. Diffusion of H^+ ions across the mucosa in exchange for Na^+ ions, and also in part accompanied by Cl^- ions (Teorell's "diffusion theory," 1933, 1947).

2. Neutralisation and dilution by the non-parietal secretion containing mucus, bicarbonate, neutral chloride and pepsin.

3. Neutralisation by ammonia derived from the action of gastric urease on urea (FitzGerald and Murphy, 1950).

4. Action of enterogastrone and urogastrone in inhibiting the parietal secretion.

5. Regurgitation of duodenal contents (Boldyreff, 1904), swallowing of saliva and naturally ingestion of food and fluid.

Teorell's "Diffusion Theory"

In Teorell's theory, first proposed in 1933, the variations in the acidity of the gastric juice depend on diffusion processes between the juice secreted at maximal concentrations and the tissue fluids or blood across the mucosal wall.

In this way H^+ ions exchange for Na^+ ions, but owing to the greater mobility of the H^+ ion and the permeability of the mucosa to chloride, H^+ and Cl^- ions likewise diffuse. The net result is a fall in H^+ ion and an increase in Na^+ ion concentration; also a fall in the molar solute concentration of the fluid. This becomes again approximately isotonic with the blood as the H^+ ion diffusion decreases with falling concentration.

The diffusion rate of the H^+ ions will naturally depend on surface, concentration, volume of gastric contents and degree of mixing.

Teorell supported his theory by experiments on anaesthetized or decerebrate cats, with the stomach ligated at each end, and the introduction of HCl in varying con-

centrations and small amounts, observations of the fluid contents being made from time to time.

His conclusions were supported by Ihre (1939) on human subjects, guarding against addition of saliva and duodenal regurgitation, also by Elliot, Risholm and Öbrink, 1942; Kaijser, 1943; Flensborg, 1944; Shay, Komarov, Siplet and Fels, 1946; and by Öbrink, 1948. Terner (1949) working on the isolated frog stomach also supports Teorell's conclusions, showing an exchange diffusion of H^+ and Na^+ ions across the mucosa.

Neutralisation and Dilution by Non-Parietal Secretion

With regard to the second mechanism, the neutralisation with dilution by a mucus secretion was advanced by Pavlov, as explaining the increase in acidity as the secretion rate increased and its decrease again as the rate died down. Hollander has strongly supported Pavlov's views in many publications (1931–1950); also Babkin (who gives a very full review, 1950).

With regard to these two theories for decrease in the primary acidity, it may be noted that both groups of supporters have accepted a high and constant acid secretion from the parietal cells. The very high primary acidity postulated by Teorell on the basis of his glycine experiments (qualified, however, by a note of caution) has already been examined in Chapter II, and a quantitative explanation advanced. From the experiments of Cope, Cohn and Brenizer with radio-active sodium (1943) as well as those of Terner and the fact that the non-parietal secretion is rich in Na^+ ions, there seems no reason to doubt apart from experimental verification that Na^+ and H^+ ions would exchange across the mucosa. The experimental findings may in part be explained by neutralisation by a non-parietal secretion, but that the rate of fall of

acidity is exponential and rapidly decreases with falling H^+ ion concentration is very significant. It is very different from what might be expected from the action of a relatively constant secretion of alkaline fluid.

Also with Teorell's glycine experiments in which the secretion into glycine solution, introduced into the stomach or experimental pouch, was examined over a wide range of secretion rate, there is no evidence of neutralisation by a non-parietal secretion. In such experiments the pH of the gastric contents was much above the normal value, and it could be argued that a low pH is necessary to stimulate the neutralising secretion.

Hollander's experiments with pouch dogs and high histamine dosage support his view that the non-parietal, or at least mucus secretion, neutralises the acidity. In these by securing an initial fast rate of flow, when this fell off later towards zero the acidity remained at high levels (see Fig. 7) Chapter II.

To interpret the maintenance of high acidity at low rates of flow as due to a previous washing out of mucus scarcely stands examination, since if such mucus secretion occurred only at a given time, its neutralising effect should be rapidly used up. It would seem that the neutralising secretion is intermittent, and liable to be cut off by high histamine dosage. However, Öbrink (1948) has advanced the most likely explanation. He points out that from the shape and size of the retention cannula a certain amount of dead space fluid, or a little "lake," is held around the head of the cannula. The newly secreted fluid must wash this out before it enters the cannula. In a steady state of secretion over a period this is unimportant, but where the rate descends from a high to a low level, over a short time, deceptively high concentrations may appear to be associated with very slow rates of flow.

It seems to follow also from Öbrink's explanation that the site of Na^+ and H^+ ion exchange, as in Teorell's "diffusion theory," is at or close to the site of secretion, and that no appreciable exchange occurs as the secreted fluid flows over the mucosa.

At the same time, there is no doubt but that the non-parietal secretion, when observable, is alkaline and contains bicarbonate in amounts more or less of the order of the plasma concentration. Evidence for this is considerable: with dogs for instance in the absence of any parietal cell stimulation, in cases of hypochlorhydria, etc.

In short the evidence for both mechanisms would appear strong and it is only a question of their relative significance.

From the analysis of Ihre's results with human subjects as given by Fisher and Hunt (1950) under Ihre's conditions at least (and these excluded saliva and duodenal fluid) it may be concluded that the magnitude of both effects amounted normally to about 10 m.eqs. of H^+ ions removed per hour.

The urease-ammonia mechanism. This, as a neutralising process, may be the most significant, judging from the extensive investigations of FitzGerald *et al.* (1946–1950) and the now established fact of the high metabolic turnover of urea and notably in the stomach (see below). It has already led to an important therapeutic application. The effect is dealt with more fully in the next chapter. A few facts here will indicate its importance.

(a) The amount of ammonia in histamine juice is proportional to the blood urea concentration (see Summary of Chapter X).

(b) It is greatly increased after the administration of urea. The gastric acidity after histamine injection is then

reduced about 40 per cent and in many instances is almost completely abolished.

(c) Urease is present in relatively high concentration in the gastric mucosa which seems to be specially rich therein.

(d) The enzyme appears in large amounts in the floor of the peptic ulcer, being much more concentrated therein than in the neighboring mucosa.

(e) The work of Leifer, Roth and Hempelmann, 1948, indicates that in the normal mouse C^{14}-tagged urea undergoes a metabolism of over 20 per cent.

More recently Hastings, Langhan, Carter and Roth (1950) have shown, using such tagged urea, the surprising fact that the whole of the body urea undergoes a metabolic turnover of 10 per cent per hour ($C^{14}O_2$ being determined in the expired air). Gastrectomy reduced this turnover.

These workers consider the possible relation to gastric acidity. That a urea mechanism is not the direct supplier of H^+ ions to the gastric juice is evident from studies on the isolated mouse stomach by Davenport and Jensen (1949) and on that of the frog by Davies and Kornberg (1950) when the acid formed far exceeds any possible change of the urea. But the facts as a whole fit well the acidic neutralising theory for the urea-urease system in the gastric mucosa, as developed by FitzGerald.

Enterogastrone and urogastrone. The inhibiting effect of fat on gastric secretion and motility has been known since the turn of the century. It was found that the effect occurs when the fat reaches the mucosa of the duodenum and upper jejunum. Lim and coworkers (Feng, Hou and Lim, 1929) showed that the effect was due to the action of an inhibitory hormone, or chalone, which they termed *enterogastrone*. Ivy and collaborators (Summary by Greengard, *et al.*, 1946) have in

recent years made preparations which have been used with limited success (100 mg. intragluteal doses) in the treatment of peptic ulcer.

Such enterogastrone preparations depress gastric secretion only moderately, and Ivy and co-workers attribute its therapeutic properties to some other factor. From the recent work of Öbrink and Winberg, using purified and relatively very active preparations, it appears that enterogastrone is not a protein. This was shown by electrophoretic studies (Öbrink, 1947), by dialysing experiments (Winberg, 1947) and the action of proteolytic ferments (Winberg and Öbrink, 1948).

These workers showed further that it gave an ultraviolet absorption curve similar to that given by purine bases (Öbrink and Winberg, 1950) and that it contained both ribose and P, indicating a nucleotide structure (Öbrink and Winberg, 1951).

It was shown to lower the secretion rate and acidity without disturbing the normal relation between these two (Hallberg, Linde and Öbrink, 1951).

Many observers have noted an effect on gastric secretion and motility similar to that of enterogastrone after the injection of extracts of urine. Gray *et al.* (1940) gave the name *urogastrone* to such an inhibitory substance extracted from normal male urine. Its properties and effects have been studied chiefly by Ivy and collaborators. It acts as a depressant of gastric secretion. Once the parietal secretion is formed neither enterogastrone nor urogastrone has any effect on its neutralisation.

SUMMARY

In a steady state the restoration of H^+ ions to the active parietal cell, removed in the oxidative phase of the redox cycle, occurs through the ionization of carbonic acid.

To supply an adequate amount the catalysis of the reaction $CO_2 + H_2O \rightleftharpoons H_2CO_3$ is necessary, and Davenport's discovery of large amounts of carbonic anhydrase in the parietal cell is briefly described.

Other cells of the epithelial wall of the gastric mucosa besides the parietal cell (which is protected by the nature of its activity) require protection from the high acidity of the gastric juice. From the nature of the cellular arrangement such cells may be assumed to be chiefly the mucus secreting cells of the surface epithelium or of the neck of the glandular tubule.

Various factors tending to diminish the acidity, once formed, are indicated. The views of Hollander and of Teorell and collaborators are briefly discussed; also the significance of the intracellular neutralising mechanism provided by the gastric urease, which has recently come into prominence through the work of FitzGerald *et al.*

CHAPTER XI

THE SIGNIFICANCE OF GASTRIC UREASE

In the previous chapter the alkaline neutralisation *within* the parietal cell was discussed, and the role of carbonic anhydrase.

Another enzyme, urease, is known to be operative in intracellular *acidic* neutralisation, the cells in this case being the neck chief cells (or mucoid cells) and the surface epithelium, in other words the mucus-secreting cells. The recent development of this subject, leading to a valuable therapeutic application, is due to FitzGerald *et al.* (1946 to 1950).

PREVIOUS HISTORY OF THE GASTRIC UREASE-AMMONIA QUESTION

Ammonia was first recognised in the stomach contents by Bidder and Schmidt (1852). Following a number of investigations into its origin and significance, Murray Luck and Seth (1924 to 1925) showed that it is formed from urea by urease present in the gastric wall. They suggested its possible significance in the neutralisation of acid.

Martin (1931, 1933) investigated the question of ammonia or urease in human gastric juice, and Rigoni (1931) the distribution of the enzyme in human gastric mucosa. Majarow (1931) examined in a roughly quantitative

manner the general distribution of the ferment in the dog and other animals. About this time Sen (1931) described a histological method (not very satisfactory) for the recognition of urease, and Vladesco *et al.* (1931) published a series of papers on the importance of the stomach in uraemia in destroying urea and eliminating the ammonia formed.

Hellerman *et al.* (1933) found that the ammonia plus urea in Pavlov pouch fluid increased proportionally with the blood urea, to which in general the urea plus ammonia in the gastric juice approximated. Reports as to increase in the ammonia in the venous blood from the stomach were conflicting (Rigoni, 1931; Mann, *et al.*, 1941).

Linderstrøm-Lang and Ohlsen (1936) studied the distribution of urease in the dog using the technique of Glick, Holter, Linderstrøm-Lang and Ohlsen (1935). They found the enzyme concentrated in the surface layers of the mucosa where cells stainable by mucicarmine are present, with none in the chief cells at the base of the glands. They found urease in the fundus and pylorus.

Rigoni (1931) also found it in the fundus and pylorus of the human stomach, and concluded it was *probably localized in the epithelial and neck chief cells.*

Recently, Glick (1949) found that the mucosa of the human pyloric antrum had little if any enzyme, but it was present in the body of the stomach, reaching a maximum in the parietal cell region. Glick considered the possibility of a relation to acid production. In a later paper this possibility was dismissed.

RECENT INVESTIGATIONS OF THE DISTRIBUTION OF UREASE

Species Distribution

FitzGerald and co-workers have shown (somewhat contrary to earlier statements of Linderstrøm-Lang and

Ohlsen, 1936; and Majarow, 1931) the presence of urease in appreciable amounts in the stomach of all the species studied—man, dog, cat, pig, rabbit, rat, mouse and frog. The most active mucosae were those obtained from man, dog and cat, the least active from the pig, rabbit and rat.

Their results pointed to the conclusion that the urease present in the gastric mucosae of the different species was roughly proportional to the acid-secreting capacity of the species. (Fossel, 1947, using dried human gastric mucosae, showed that the whole stomach could form from urea 60–120 m.eqs. ammonia/hr.)

Tissue Distribution

Small amounts of urease were found in many tissues, but according to Table X, the content of the enzyme in the gastric mucosa exceeded that of any other organ or tissue.

TABLE X

Urease Activity of Tissue Expressed as Micrograms Ammonia-N Released per G. of Wet Tissue per Hour at 37° C.

Animal	Stomach	Liver	Kidney	Pituitary	Adrenal
Dog (1)	3,160	136	...	270	200
Cat (2)	1,210	376	355	...	...
Mouse (11)	2,860	35	69	...	...
Rat (3)	296	27	12	...	...
Frog (6)	201	17	34	...	...

Gastric Distribution

The highest concentration occurred in the cardiac mucosa, less in the pylorus and much less in the duodenum. With respect to the distribution in depth it could easily be shown from scrapings of the mucosa that the enzyme was concentrated in the superficial layers, which agrees with the findings of Linderstrøm-Lang and collaborators.

Such distribution might be related to increasing sensitivity of the mucosa to acid on passing from cardia to duodenum (Bachrach, Grossman and Ivy, 1946).

DEMONSTRATION OF THE FUNCTIONAL ACTIVITY OF GASTRIC UREASE IN MAN

From the above and considering the distribution of the enzyme and its capacity to produce ammonia, it would appear not improbable that it was active in neutralising gastric acidity. The following evidence shows that for the human subject it is, in fact, a major factor in neutralisation.

Experimental procedure. The stomach of the fasting subject was emptied, the contents kept for analysis, as well as a blood sample. Sixty milliliters of a solution of acacia flavoured with orange was then introduced into the stomach, histamine acid-phosphate (0.01 mg. kg. body weight) being injected at the same time subcutaneously. After the injection, the stomach was emptied as thoroughly as possible every 20 minutes up to one hour, the contents being measured and analysed later. A blood sample was then taken and the subject given another 60 ml. of the solution as before but containing 15 g. of urea. At the same time histamine was again injected and the gastric fluid removed as before up to one hour when a final specimen of blood was taken.

In this way 14 normal subjects, 10 gastric ulcer and 25 duodenal ulcer cases were investigated. Some experiments were also conducted in which the urea was injected intravenously instead of being orally administered.

The results may be expressed as follows.

(1) The output of acid after the second histamine injection falls markedly when urea is administered. It does not fall, if urea is withheld.

In 55 cases examined, there was a fall in 53, and a slight increase in two cases, which latter may be explained in terms of sample variability.

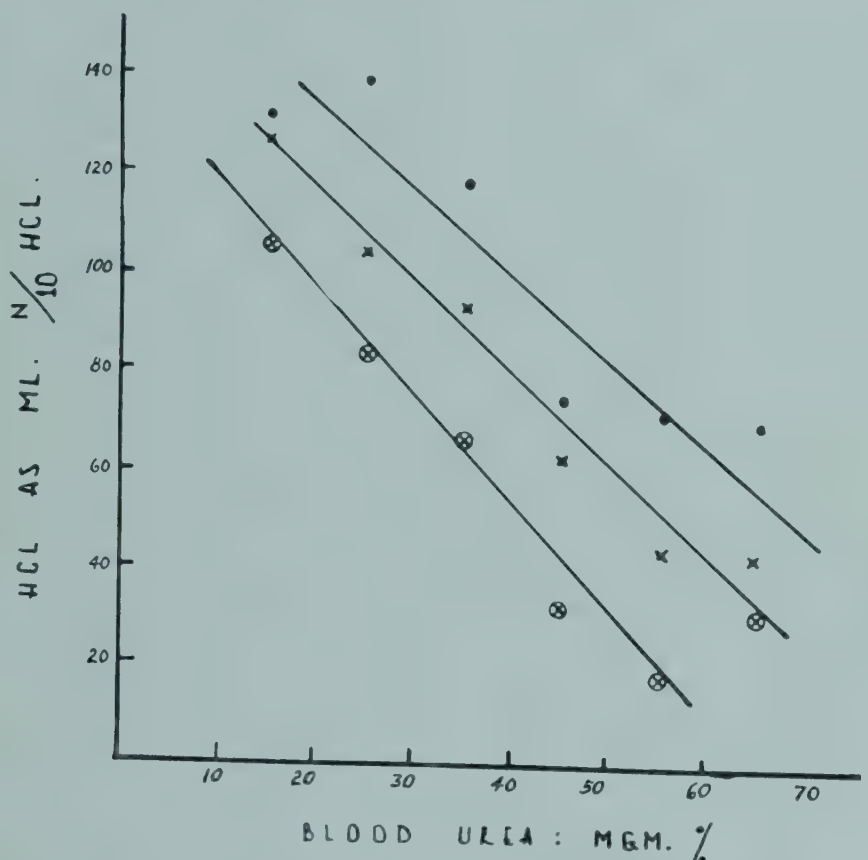

Fig. 25. Graph of mean ml. N/10 HCl over various ranges (10 to 19, 20 to 29, etc.), of mg. blood urea concentration. × = total data (49 cases). ● = duodenal ulcer group (25 cases). ⊗ = remainder (24 cases), i.e., normal and gastric ulcer cases. (Courtesy of *Irish J. M. Sc.*)

The total mean fall was 42 ml. of N/10 acid or close to 40 per cent of the acid secretion without urea.

The mean fall for the 20 normal subjects was 33 ml. N/10, for 25 duodenal cases 53 ml. N/10, and for 10 gastric ulcer cases 34 ml.

TABLE XI

MEAN RESULTS OF THE SALIENT FEATURES OF THE DOUBLE HISTAMINE TEST WITH UREA

Subject	Number of objects	Volume (minus the 60 ml. introduced)		HCl as ml. N/10		Cl as ml. N/10		Ammonia-N as ml. N/10		Blood urea change mg. per cent	
		1	2	1	2	1	2	1	2	1	2
Normal	14	90	133	83	55	150	179	5·4	22·2	28	40
Gastric ulcer	10	102	124	61	27	162	162	10·7	45·6	33	45
Duodenal ulcer	25	144	165	148	95	216	218	6·5	22·2	27	40
Whole series	49	120	147	111	70	186	196	7·0	27·0	29	41

Figure 1 at top of column refers to results obtained in preliminary control period of one hour. Figure 2 at top of column refers to results obtained in period of one hour after urea.

The second histamine test, when carried out without urea in six normal subjects showed no reduction in the acid secretion over the first (within the sampling error) which agrees with many other observations in the literature. The results are summarised in Table XI. Figure 25 also shows the mean value of the HCl output plotted against the blood urea.

This great effect of urea administration does not arise from some action on the parietal cells, either directly or by inhibition of the action of histamine. In support of such conclusions one may note:

(a) There is no correlation between the fall and the previous output of acid.

(b) There is no reduction in secretion of water or of chloride, but rather an increase in both, which may be explained by some abstraction of fluid by osmosis.

It may be added that the marked fall in acid secretion after the histamine injection is also evident if the urea is

injected intraveneously instead of being administered orally.

(*2*) *A marked increase of ammonia appears in the gastric juice after urea administration.*

If the acid secretion in the second hour (after urea) be subtracted from that in the first hour, and similarly with the ammonia, the first hour levels may be taken as means and the correlations examined between such subtractions or deviations. (These correlations should be less than the true values since the secretion in the first hour is not a fixed constant.) Proceeding in this way, the following correlation coefficients (r) have been obtained:

For 14 normal cases, $r = -0.60 \pm 0.17$.
For 10 gastric ulcer cases, $r = -0.73 \pm 0.14$.
For 25 duodenal cases, $r = -0.61 \pm 0.12$.

The regressions for these deviations are:

$$\text{Am.} = -0.48\ \text{Ac.}$$
$$\text{Am.} = -0.72\ \text{Ac.}$$
$$\text{Am.} = -0.21\ \text{Ac.}$$

or, for the normal subjects the fall in acid is accompanied by about half its equivalent rise in ammonia. For the gastric ulcer cases there is proportionally more ammonia and for the duodenal ulcer cases there is less. Figure 26 shows the mean values of the ammonia content of gastric juice (after histamine) plotted against the blood urea. The observations include those with and without urea administration.

(*3*) *Without urea administration the gastric juice after histamine contains a mean level of ammonia which is proportional to the concentration of blood urea.*

The correlation coefficient of the ammonia of the gastric contents, for the hour after the first histamine injection, with the blood urea (total of 49 obs.) was found to be 0.58 ± 0.09. The regression equation is:

$$\text{Am.} = 0.35\text{B} - 2.1$$

The ammonia formed is given in ml. N/10 ammonia with B (the blood urea concentration) expressed in mg. urea/100 ml.

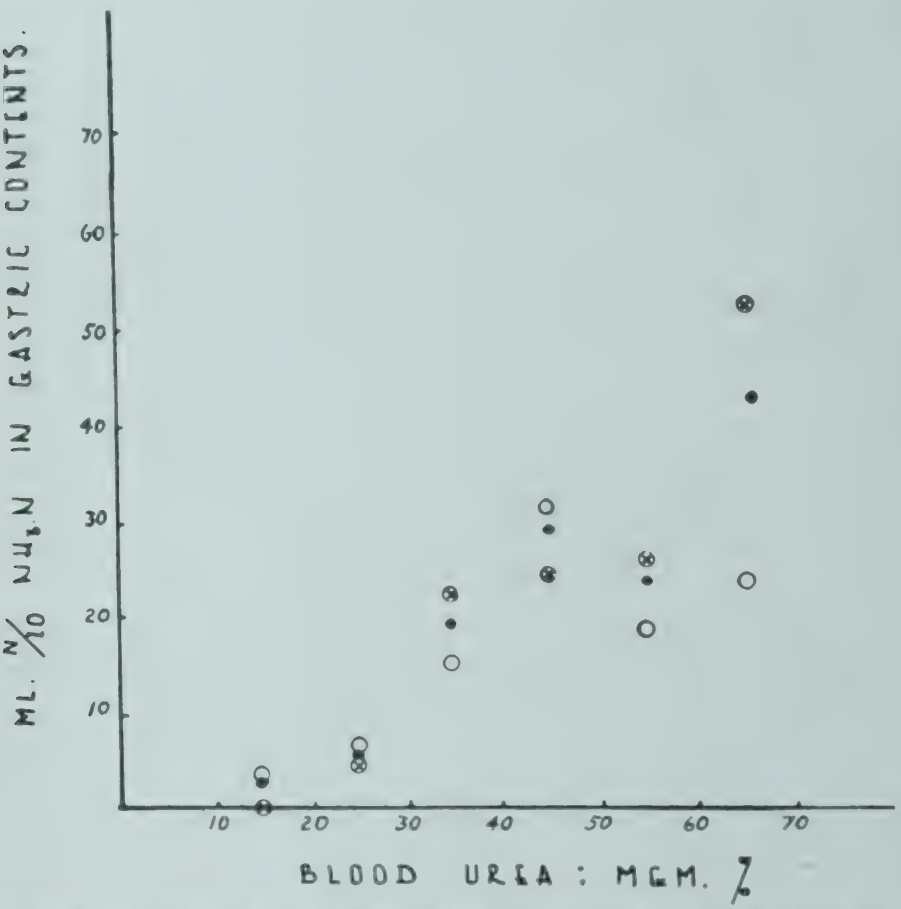

Fig. 26. Graph of ml. N/10 NH_3-N in gastric contents plotted against blood urea before and after urea. The mean ammonia-N value for each range of blood urea concentration has been inserted in a manner similar to Figure 25. ● = total data (49 cases). ○ = duodenal ulcer group (25 cases). ⊗ = remainder (24 cases), i.e., normal and gastric ulcer cases. (Courtesy of *Irish J. M. Sc.*)

(4) *The gastric mucosa contains more than enough urease to neutralise the total acid formed after histamine stimulation.* FitzGerald (1946) showed this for the cat, and Fossel

(1947) has shown the same for man. From the above it will appear then that the urease-ammonia mechanism in man is significant in neutralising the acidity of the gastric juice.

ANIMAL EXPERIMENTS

Besides the human subject there have been only a limited number of animal experiments directly bearing on this problem. They may be divided into two groups.

(a) **Partially isolated stomachs,** using either cats (FitzGerald and Murphy, 1950) or frogs (FitzGerald, 1950). The stomach with its circulation was left in situ in the body but it was separated from the oesophagus above and the duodenum below by suitable ligatures. In the cat experiments acid was instilled into the isolated stomach and its neutralisation followed.

In the majority of cases neutralisation was slow but in a small number it was very rapid with ammonia being formed. In the frog experiments (FitzGerald, 1950), referred to above, HCl was instilled into the isolated stomachs of pithed frogs accompanied by a small injection of saline. After one hour the pH and ammonia content of the fluid were determined. There was always some fall in acid strength with increase in ammonia concentration.

(b) **Completely isolated stomach.** This well-known technique was first described by Delrue (1935). His arrangement and that of Gray (1943) are shown in Figures 27 and 28. Davenport (private communication, 1950) has shown that the technique for the isolated stomach can be greatly improved over that hitherto used and with quantitatively different results. With respect to the present discussion though Davenport and Jensen (1949), had found that added urea had but little effect on

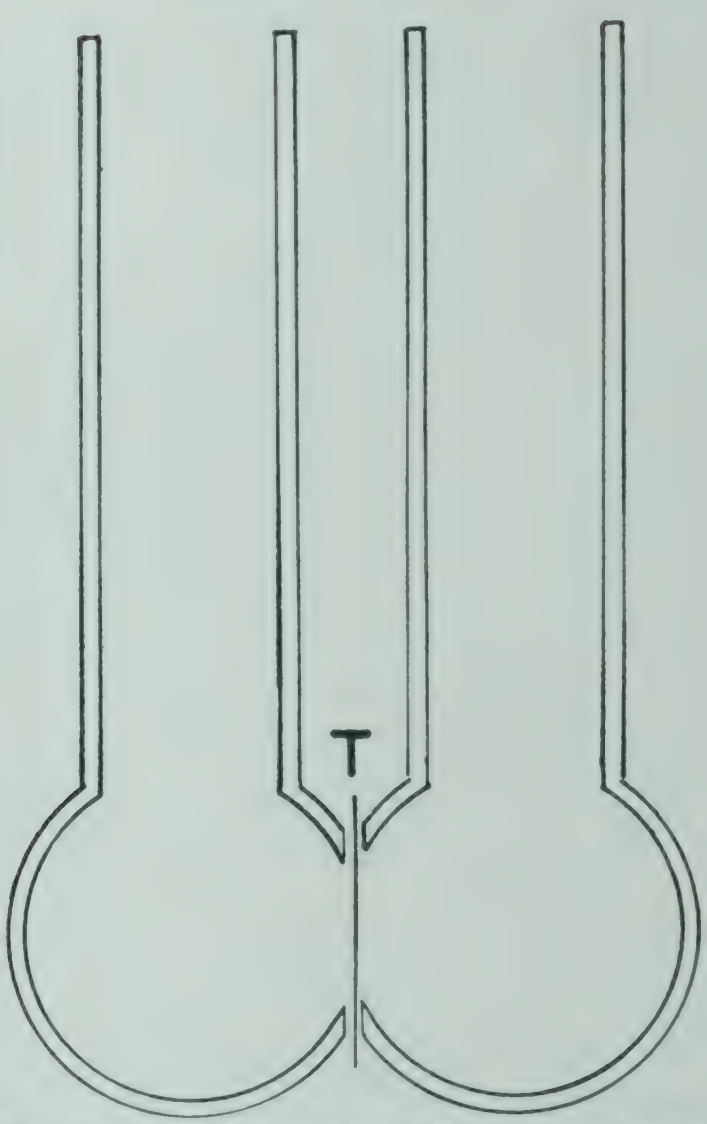

Fig. 27. Delrue's apparatus (1935) for investigating acid production by the isolated gastric mucosa of frog. The mucosa is protected from the glass by rubber rings with central aperture of 8 mm. diameter.

the secretion of acid by the isolated mouse mucosa; with his new technique he finds that urea has a marked depressing effect whether the stomach is secreting spontaneously or in response to carbaminoylcholine.

It will appear therefore that taking account of this as well as FitzGerald's results with the partially isolated frog stomach, no significance may be attached to the finding of

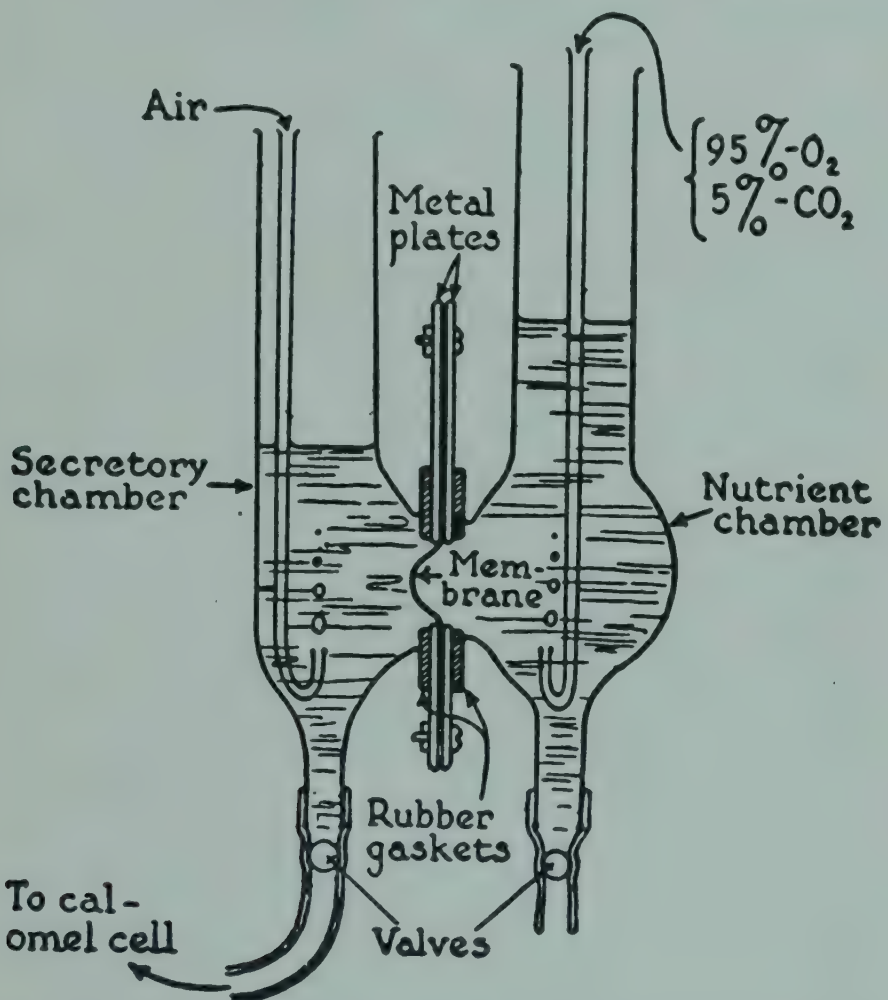

Fig. 28. Gray's apparatus for studying the in vitro secretion of acid by the gastric glands of the frog. (Courtesy of *Gastroenterology*.)

Davies and Kornberg (1950) that urea added to the isolated frog's mucosa was without effect on the secretion of acid. It may be added that the recent paper of Glick *et al.*

(1950), provides further evidence against the conclusions of Davies and Kornberg (1950).

AN HYPOTHESIS CONCERNING THE RELATION OF THE ACID NEUTRALISING MECHANISMS

The parietal cell, secreting a high concentration of HCl, is by the nature of this mechanism protected against HCl. Further, as the pure secretion contains only H^+ and some K^+ ions, it may be supposed that the cell is impermeable to Na^+ ions. Apparent evidence to the contrary is connected with the whole mucosa.

The mucoid cells of the neck and the surface epithelium, which come more closely into contact with the main acidic secretion, are, on the other hand, not protected by the nature of their activity. As they can secrete Na^+ and HCO_3^- ions, it may be assumed that H^+ and Cl^- ions can enter these cells rather freely.

In the Hollander mechanism HCO_3^- ions are represented as being secreted outwards with Na^+ from mucus secreting cells even at the height of the parietal acidic secretion. It would be more rational to suppose here that an actual HCO_3^- ion secretion *outwards* occurs only when the parietal secretion is very low or absent, but that otherwise the H^+ ions enter the mucus cells and neutralise the HCO_3^- ions therein. The H^+ ions can be represented as diffusing in with HCl or exchanging for Na^+ ions, which is the essential feature of Teorell's diffusion theory.

The Teorell process, though reducing the intragastric acidity, transfers acid into the surface and mucoid cells. For these cells it is not a neutralisation mechanism, but on the contrary an acidifying process.

The ammonia mechanism comes now into play (Fitz-Gerald's theory).

$$OC\begin{matrix}NH_2\\ \\ NH_2\end{matrix} \rightarrow 2NH_3 + CO_2$$

At the pH of the cell the CO_2 largely escapes. H^+ ions are taken up by the NH_3 molecules:

$$NH_3 + H^+ \rightarrow NH_4^+$$

the fixed buffers releasing H^+ ions, and developing a larger number of negative charges. In other words, the cell becomes more alkaline and is restored towards its resting pH.

The NH_4^+ ions now pass in two directions, exchanging with H^+ or Na^+ ions from the gastric juice, and with Na^+ ions in the interstitial fluid or plasma. The net result is that the NH_4^+ ions so appearing in the gastric juice are not a measure of the degree of neutralisation that has occurred in the cells. A large fraction may have passed into the blood. The process is roughly indicated in Figure 29 where only the exchanging ions are shown, and with no implication made as to concentration or the like.

UREA THERAPY OF THE PEPTIC ULCER

Urea has certain theoretical advantages over the usual antacids.

1. It forms part of the physiological protecting mechanism which may be at fault in the peptic ulcer.
2. Its rate of emptying from the stomach is unimportant, as it does not act as an antacid until changed to ammonia in the mucosa after absorption.
3. No disturbance of the acid-base balance can occur as the absorbed ammonia will be carried to the liver and rapidly re-formed into urea (Koprowski and Uninski, 1939).

At the same time it may be noted that therapeutic doses may be unpalatable, that definite renal damage is a contra-

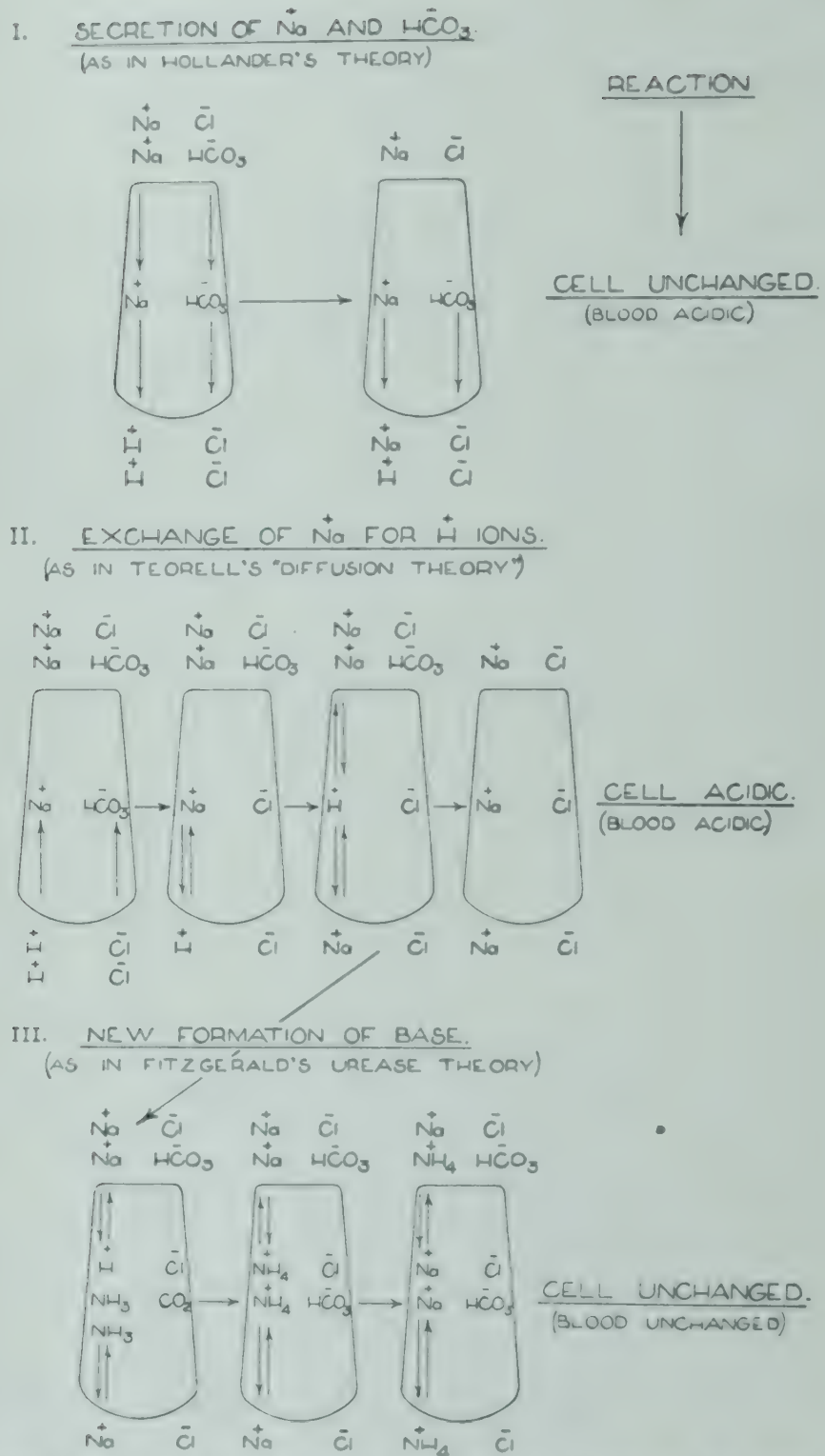

Fig. 29. The above diagrams suggest the nature of the more significant ionic interchanges occurring. No quantitative relations are implied.

indication, and that blood urea determinations should be carried out at intervals at the beginning of treatment, especially in older patients.

Urea solution. To produce the required and persistent rise in blood urea it is necessary to give doses of about 15 g. of urea in suitable solution or mixture. This amount of urea requires about 30 ml. of water, and even then it will not always remain in solution at room temperature, especially in cold weather. At such times it is best kept warm before use. Acacia is used to stabilise the solution and help in disguising the taste. With the same object various syrups or liquorice have been used, but not with marked success, though further investigation might well improve the mode of administration.

Dosage. The usual dosage was 75 g. per day, but in the initial period 90 or even 120 g. per day may be required. With older patients, a lower level (60 or even 45 g. per day) was often sufficient. Patients are usually advised not to increase their fluid intake during treatment, as some fluid restrictions help to maintain the level of blood urea.

Selection of cases. The most suitable cases for investigation were those ulcer patients suffering from persistent dyspepsia resistant to the usual standard medical treatment. Nearly all the cases examined had received either a simple gruel test meal or a histamine test, especially the double test with urea.

In general, the better the response to urea in the double histamine test the better were the therapeutic results; but it was not always so, and a poor response might be occasionally followed by a good result in treatment, and vice versa.

Barium meal examination was carried out in every

case, and it was sought to have at least one film taken during treatment.

As part of the diagnostic measures occult blood examination of the faeces and gastroscopy were often carried out. Levels of blood urea were determined in the great majority of cases before and during treatment, and accompanied, when advisable, by urinary examination and renal function tests.

Gastric ulcer cases were selected for this treatment either because they were known to have had an ulcer for

TABLE XII

Summarized Results of Urea Treatment of 79 Patients Suffering from Various Forms of Peptic Ulcer

Diagnosis	Excellent	Good	Fair	Poor	Total
Gastric ulcer	12	6	3	0	21
Duodenal ulcer	29	10	6	2	47
Anastomotic ulcer	7	3	0	1	11
Totals	48	19	9	3	79

Excellent = The patient is quite symptomless either on continued treatment or none at all.
Good = The patient is very definitely improved, but not free of symptoms.
Fair = Some temporary relief from urea.
Poor = No relief of importance noted.

many years or because a newly recognised ulcer was unmistakably benign.

In general the intention was to select for urea therapy patients who had ulcer symptoms and signs refractory to standard medical measures. The great majority of the patients were ambulant. In a small number of cases a control solution was used after a period of urea therapy.

Results. The results of the urea treatment of 79 patients suffering from various forms of peptic ulcer are given in Table XII, from which the efficacy of the treatment may be judged.

It may also be remarked that the development of fresh ulcer symptoms in a number of patients on the control solution, who had previously done well on the urea therapy, lends additional support to the efficacy of the treatment.

The additional Table XIII is here of much interest. It shows the amounts of urease found in superficial and deeper scrapings of the mucosa of a typical peptic ulcer and of its surrounding mucosa; also of that at some distance. It will be seen that there is much increased concentration of urease in the superficial floor of the ulcer,

TABLE XIII

UREASE IN THE PEPTIC ULCER

Site of mucosa	Urease units	
	Superficial scrapings	Deep or remaining mucosa
Ulcer	55,000	17,400
Around ulcer	25,500	18,200
At some distance	18,200	19,000

Determinations of urease in the superficial and deep mucosa of a peptic ulcer, compared with those in the surrounding mucosa and that at some distance. Urease expressed as micrograms ammonia-N released per g. of wet tissue per hour at 37° C.

when compared to the healthy tissue, and in the light of the foregoing account the functional significance of this increase is strongly suggested.

Conclusions. The results to date show that the urea therapy gives good results, particularly with patients having very persistent dyspepsia. It is of special value in the treatment of gastro-jejunal or anastomotic ulcers.

Follow-up data are quite inadequate for assessing the treatment with respect to relapse, but it may be stated that some cases successfully treated three years ago and under present observation are still in remission.

SUMMARY

Evidence amounting to proof is given (from FitzGerald and Murphy, 1950) that the gastric urease in the human subject has a functional significance in neutralising gastric acidity and protecting the mucosa. Corroborative evidence is given for other animals.

Thus, it has been shown that after urea administration sufficient to raise the blood urea level about 12 mg. per cent, there is a mean fall of acidity in the gastric juice after histamine injection, amounting to 40 per cent. About half the neutralising ammonia appears in the gastric juice; the remainder, presumably, enters the blood stream. The effect cannot be explained by a depression of the parietal secretion.

Without urea administration there is a significant positive correlation ($+ 0.58 \pm 0.09$) between the level of blood urea and of ammonia in the histamine juice.

There is more than enough urease in the gastric mucosa to neutralise the whole of the parietal secretion, and the concentration of urease in the gastric mucosa greatly exceeds that in other tissues.

Urease is specially concentrated in the floor of the peptic ulcer, as compared with the neighbouring healthy mucosa.

An hypothesis is advanced as to the manner in which the urease mechanism acts, and considered in relation to the theories of Teorell and of Hollander.

The urea therapy of the peptic ulcer has been shown to be a valuable therapeutic procedure, and particularly so in the treatment of ulcer cases with dyspepsia resistant to standard medical treatment.

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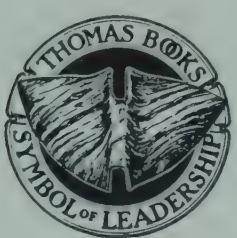

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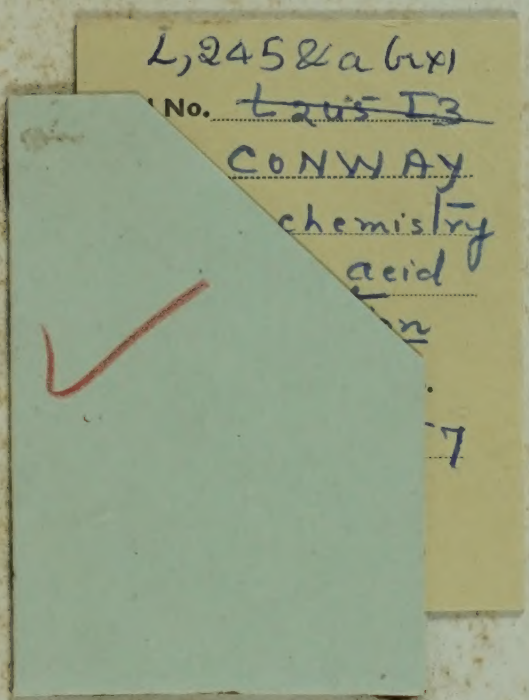
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